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APHIS REPORT OF ACCOMPLISHMENTS IN ANIMAL PRODUCTION FOOD SAFETY FY 1995

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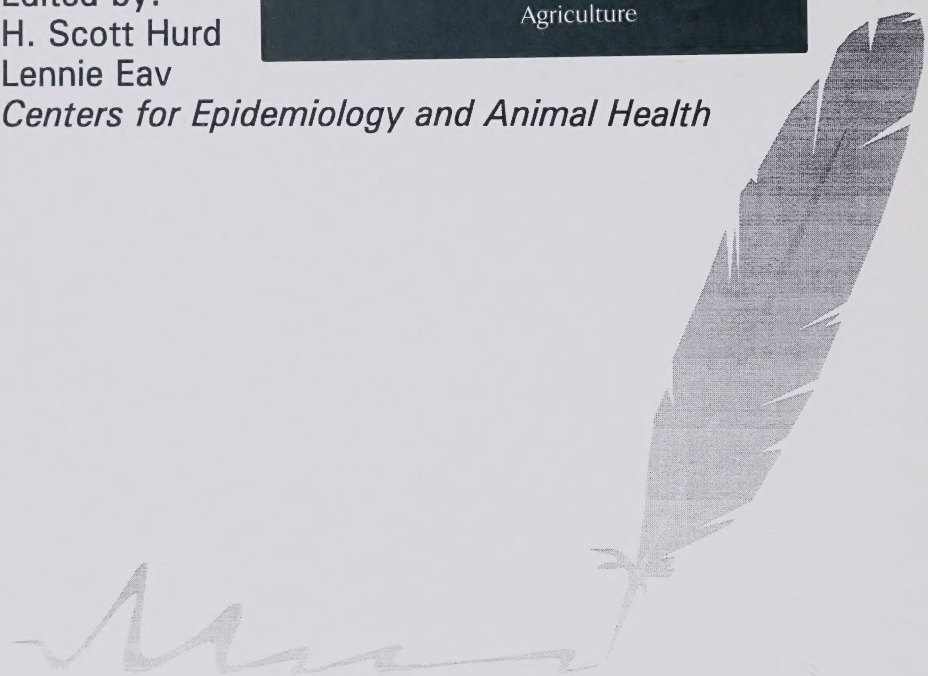

Executive Summary

From the beginning of the brucellosis and tuberculosis control programs, APHIS has had a critical role in animal production food safety. During FY 1995, APHIS continued efforts in this arena under FSIS authority, with a variety of field research, training and traceback activities. Many of the projects were successful only because of close collaboration and cooperation with universities, industry, the Agricultural Research Service (ARS), and the National Agricultural Statistics Service (NASS). This report contains preliminary results of animal production food safety studies conducted under the aegis of APHIS.

Only three years after the major *Escherichia coli* O157:H7 outbreak in the Pacific Northwest, we have acquired some information about the ecology, source and potential control factors for *E. coli* O157:H7 in dairy cows. We have clear evidence that *E. coli* O157:H7 can colonize multiple animal species on a farm. It was isolated from one horse, two dogs, flies, and a pooled bird droppings sample. The organism was also isolated from water trough samples and water trough biofilm. The percentage of infected dairy herds in the Pacific Northwest is 75% or greater. Within-herd prevalence is low, below 5%. Several nutritional variables were found to be associated with the prevalence or presence of *E. coli* O157:H7. Also, horizontal transmission between calves appears important.

We now have an early indication of *E. coli* O157:H7 prevalence in swine, sheep, feedlot cattle, and cull dairy cows. At this point, we have tested a total of 2,226 fecal samples from grower/finisher hogs. None were positive for *E. coli* O157:H7. Two studies, one of auction sheep in New Jersey and the other of slaughter sheep in the Midwest, found low *E. coli* O157:H7 prevalence (0.4% and 0.9%, respectively). In a 13-state study of feedlot cattle in 100 feedlots, we detected *E. coli* O157:H7 in 63% of the feedlots but detected only 1.6% of samples. An ongoing study in New York state estimated a prevalence of about 1.0% in cull dairy cows.

Salmonella enteritidis (SE) prevalence information, critical for decision making, was generated in 1990 and again in 1995 from the spent hen and liquid egg surveys. These data show that the prevalence of SE in liquid eggs has nearly doubled in the Northeast and the West since 1990. The overall prevalence of SE was 19%. In spent layer hens at slaughter, 45% of flocks had one or more SE-positive birds. Results of this survey suggest an increase, or at least no decrease, in the U.S. flock prevalence since 1990.

The Pennsylvania *Salmonella enteritidis* pilot project began in APHIS in 1992. Analysis completed in 1995 yielded critical information regarding the epidemiology of SE in layer flocks. The egg positivity rate was found to be around 2.75 eggs per 10,000. Findings of this project suggested risk factors for SE, including mouse infestation, molting, and increased flock age. Analysis of the sampling protocols gave helpful information about detecting SE in flocks. For example, blood spot eggs are more likely to be SE positive than nest run eggs. Flocks with greater than 50% positive manure sample were more likely to have positive eggs. Testing of manure pits provides a better measure of SE status than egg belt testing. Information from this pilot project was used to develop the industry-led Pennsylvania Egg Quality Assurance Program.

In California, APHIS helped industry with the development of California's egg quality assurance program. This was based on a successful experience in developing a dairy beef quality assurance program.

We described the shedding of all Salmonella serotypes in cattle feedlots. In 100 feedlots from the 13 major cattle feeding states, Salmonella was detected in 38% of the feedlots. Of the pen floor fecal samples collected, 5.5% were positive. The Salmonella serotypes did not closely match those found in reported human cases of salmonellosis.

APHIS is working with the pork industry to quantify the trichinae problem and explore available testing methods. Currently, over 5,000 on-farm samples have been tested. Preliminary analysis indicates that the U.S. swine herds still have low levels of trichinae infection, that waste feeders are not more likely to be infected, and that the Enzyme-Linked Immunosorbent Assay (ELISA) test is very sensitive in detecting trichinae.

The APHIS Epidemiology Delivery System responded enthusiastically and contributed substantively to the animal production food safety information needs. This response was made possible through the coordinated efforts of the APHIS field force, NASS, APHIS liaison at Centers for Disease Control and Prevention (CDC), the National Veterinary Services Laboratories (NVSL), and the Centers for Epidemiology and Animal Health (CEAH).

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Escherichia coli O157:H7

Sources of Verotoxic *Escherichia coli* O157:H7 in Feedlots and Dairy Farms in the Pacific Northwest

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Abstract

The purpose of this study is to determine if: (a) *E. coli* O157:H7 can be found in feces of non-bovine species on or in the vicinity of a cattle farm, (b) feeds brought into farms contain *E. coli* O157:H7, (c) samples collected from water troughs contain *E. coli* O157:H7, and (d) *E. coli* O157:H7 isolated from non-bovine species is distinguishable from subtypes found in bovines on the same farm. Four herds each (two dairies and two feedlots) were selected in Idaho, Oregon, and Washington, for a total of 12 herds. Livestock on the contiguous properties were sampled by collecting swabs of fecal pats or single fecal pellets. Samples were collected from bovine, other livestock, dogs, cats, birds, rodents, and flies. In addition, water troughs and feed samples were collected. *E. coli* O157:H7 positive cattle were identified on all 12 herds with prevalence ranging from 1.5% to 6.1% in feedlots to 1.1% to 4.4% in dairies. *E. coli* O157:H7 was found in fecal samples collected from one horse and two dogs. It was also detected in a pooled bird droppings and in two composite fly samples. *E. coli* O157:H7 was not detected in samples from rodent, cats, or wildlife. Four (1.3%) out of 327 trough water samples and six (2.0%) of 320 water trough biofilm samples yielded *E. coli* O157:H7. This study provides clear evidence that *E. coli* O157:H7 can colonize multiple species on a farm. It remains for future studies to determine which of these species, if any, is the reservoir of the *E. coli* O157:H7.

Purpose

Previous studies indicate that although *E. coli* O157:H7 exists in most cattle operations, the prevalence is highly variable among herds. Furthermore, the shedding of *E. coli* O157:H7 appears to be strongly clustered temporally within high prevalence herds. For example, herds will be negative for *E. coli* O157:H7 for months or years and suddenly have a burst of culture positives. These data suggest the presence of a reservoir for *E. coli* O157:H7 external to bovines. The molecular subtyping data from our previous studies provide further evidence of a reservoir for *E. coli* O157:H7 external to bovines. Multiple subtypes exist simultaneously on a single farm. Some subtypes come and go within a few months; others persist for years. Indistinguishable subtypes exist on farms hundreds of kilometers apart. It seems possible from these data that one or more species other than cattle represent a source of *E. coli* O157:H7. It also seems possible that environmental sources (feed or water) may play a role in inter- and intra-farm transmission.

The purposes of the present study were to determine if:

1. *E. coli* O157:H7 can be found in feces of non-bovine species on or in the vicinity of a cattle farm.

2. Feeds brought onto farms contain *E. coli* O157:H7.

3. Samples collected from water troughs contain *E. coli* O157:H7.

4. *E. coli* O157:H7 isolated from non-bovine species subtypes are indistinguishable from that found in bovines on the same farm.

Materials and Methods

Experimental Design

Four herds were selected in each of the three states (Idaho, Oregon, and Washington), for a total of 12 herds. Two herds from each state were dairies and two were feedlots. Sampling was scheduled to occur on three occasions separated by approximately one month. The first sampling trips were made to each herd in July or August and the last trips occurred in September or October. Additional sampling trips were necessary beyond the three scheduled in order to collect sufficient numbers of non-bovine samples. In general, these trips were interspersed with scheduled visits, though a few occurred through mid-November.

Types of Samples

Bovine - For dairies, 60 fresh fecal pats were sampled from pens containing heifers aged 6-24 months. Sample collection was equally distributed among all pens containing animals in this age group. For feedlots, 10 fresh fecal pat samples were collected from each of six pens: the three pens shortest on feed and the three pens longest on feed. Fecal pats were sampled by inserting a cotton-tipped swab, rotating, shaking off excess feces, then placing the swab into a tube containing enrichment media.

Other livestock - Livestock on the same contiguous property were sampled by collecting swabs of fecal pats or single fecal pellets (depending on the form of the stool).

Wild animals - Fecal droppings were collected from wildlife species that visited the farms frequently. Collection of wildlife samples took at least 5 hours per farm. Pelleted feces were collected as single pellets; soft feces were sampled using swabs.

Dogs and cats - Swabs of recently passed stools or rectal swabs.

Bird feces - Pooled samples of droppings and other intestinal samples.

Water troughs - Up to a maximum of 10 troughs were sampled in each farm. Where there were more than 10 water troughs, samples were collected from troughs in pens that contained animals from which fecal samples have been collected. Water and biofilm samples were collected from each selected water trough. Samples (at least 50 ml) were collected by placing the mouth of a sterile plastic sampling jar at the surface of the water.

Feed samples - Prior to mixing, all component feeds were sampled from each farm. Feeds were collected from locations where they were unlikely to have been environmentally contaminated; thus, feed contaminated with *E. coli* O157:H7 would have come into the farm with the feed. At least 50 grams of each feed was collected and placed in a ziplock bag. New exam gloves were used each time feed samples were collected. In addition to component feeds, feeds from two feed bunks in the feedlots were collected. Samples were collected from pens on feed the longest and pens on feed the shortest time.

Rodent samples - Live traps were used to collect rodents. Twelve traps were set in feed storage areas and other areas suspected to be runways in each farm. Some rodents were captured directly from nesting areas (not in traps).

Captured rodents were euthanized using carbon dioxide gas, placed in whirl packs, and transported to the laboratory on ice.

Fly samples - The contents of two or more fly traps were collected per farm per sampling visit. A small jar of autoclaved ground beef was used as an attractant. Traps were left hanging for at least five days. Captured flies were killed using a blast of household insecticide, placed in a whirl pack, and transported to the lab on ice.

Laboratory Methods

Fecal pat and rectal swab samples (cattle, other livestock, cats, dogs, wildlife) - Samples were collected using cotton tipped swabs evenly coated with fecal matter and placed into screw-capped tubes containing 3 ml of tryptic soy broth (Difco Labs) containing 40μg/ml vancomycin (Lyphomed) and 50 ng/ml cefixime (American Cyanamid) (TSBcv). If feces were pelleted, then one pellet was crumbled into eight mls of TSBcv. Samples were then placed on ice and sent to the Field Disease Investigation Unit (FDIU) research laboratory via overnight delivery. Upon arrival at the laboratory the tubes were briefly agitated, incubated at 37°C for approximately 16-24 hours and then serially diluted to 10^{-4} in 96 well plates using TSB as the diluent. For each sample 300 μl of both a 10^{-3} and 10^{-4} dilution were evenly spread onto separate 150 mm sorbitol MacConkey agar plates (Difco Labs) containing 50 ng/ml cefixime and 2.5 μg/ml potassium tellurite (SMACct) and then incubated at 37°C for 16 -24 hours. Up to 10 sorbitol non-fermenting colonies per sample were transferred to MacConkey agar plates to test for lactose fermentation. Sorbitol negative lactose positive colonies were then tested for beta-glucuronidase activity in a 96 well plate assay which incorporated 80 μl per well of TSB with 100 μg/ml 4-methylum-billferyl-B-D-glucuronide (MUG). The MUG plates were incubated overnight at 37°C and then examined on an ultraviolet transilluminator. All sorbitol negative, lactose positive, and MUG negative colonies were assayed for *E. coli* O157:H7 antigen using a commercially available latex agglutination kit (Oxoid, USA).

Composite Bird Fecal Samples - Fecal samples were collected in whirl pak bags using a sterile tongue depressor, placed on ice and sent to the FDIU laboratory. At the laboratory, 10 ml of TSBcv were added to each sample and then cultured as described in the methods for fecal samples, except that samples were plated at the serial dilutions 10^{-2} and 10^{-3}.

Live trapped rodents - Up to 10 fecal pellets from each rodent were extracted using a sterile scalpel and forceps and cultured following the method described for fecal samples.

Fly trap samples - To each fly trap composite sample, 10-50 ml of TSBcv (dependent upon the number of flies) were added to each bag and then homogenized using a stomacher homogenizer. The samples were then cultured as described for fecal samples.

Trough water samples - For each sample, 30 ml of trough water was transferred into a sterile specimen cup and combined with 30 ml of a 2X concentrate of TSBcv was mixed and incubated overnight at 45°C. The samples were then cultured as described for fecal samples except plating was done at the serial dilutions 10^{-4} and 10^{-5}. If there was minimal growth on the SMACct plate, the samples were replated using less serial dilution.

Trough biofilm samples - Depending on the volume of sample, 20-50 ml of TSBcv were added to each sample, homogenized, and incubated at 45°C for 16-24 hours. The samples were then cultured as described for fecal samples except plating was done at the serial dilutions of 10^{-4} and 10^{-5}. If there was minimal growth on the SMACct plate, the samples were replated using less serial dilution.

Cattle feed samples - For each sample, 15 gm of feed were placed into a sterile specimen cup and then mixed with 60 ml of TSBcv. Samples were incubated at 45°C for 16-24 hours. The samples were then cultured as described for fecal samples. If there was minimal growth on the SMACct plate, the samples were replated using less serial dilution.

Laboratory methods - Pulsed field gel electrophoresis (PFGE) was performed following a modification of the method described by Barrett, et al.

Results

The six study feedlots ranged in size from 250 to 14,750 total animals with a median of 1,550 animals. The six dairies ranged in size from 290 to 1700 total animals of all ages with a median of 904 animals. Domesticated species other than bovines were present on all of the other farms (Table 1). All farms reported frequently sighting a variety of birds in their farms (Table 2).

Table 1. Number of Non-Bovine Species Per Farm[a]

Species	Feedlots			Dairies		
	n>0	Median	Range	n>0	Median	Range
Horses	6	8	1-12	2	0	0-5
Sheep	0	-	-	2	0	0-10
Hogs	0	-	-	1	0	0-7
Goats	0	-	-	0	-	-
Dogs	1	0	0-4	6	3	2-5
Cats	4	3.5	0-12	5	10	0-20

[a] *As reported by owners of study farms at the beginning of the study period.*

Table 2. Number of Farms Reporting Bird Sightings

Bird Type	Feedlot	Dairy
Starlings	6	6
Pigeons	6	5
Seagulls	3	1
Ducks	6	2
Geese	6	5
Sparrows	6	5
Swallows	5	4

Fly, bird, and rodent control efforts reported by owners are shown in Table 3. Two feedlots and three dairies used insecticides for fly control. Four feedlots and three dairies reported active rodent control efforts (traps or poison). Four feedlots and three dairies reported active bird control efforts. Controlling birds with firearms was the method of choice reported by the owners.

Table 3. Number of Farms Reporting Bird, Fly, or Rodent Control

Control Method	Feedlot	Dairy
Fly and bird control		
Use insecticides for fly control	2	3
Active bird control (firearms only method used)	4	3
Rodent control		
Trap	1	2
Poison	4	3
Trap and/or poison	4	3
Cat	2	3

All herds used water troughs or automatic waterers. For the 4-24 months age group from which fecal samples were collected, all used water troughs. In one herd, cattle had direct access to surface water. Water trough management practices in the study herds are shown in Table 4.

Table 4. Factors Related to Water Trough Management in Study Herds

Trough Management Factors	Feedlot	Dairy
Trough cleaned in past 3 months	3	3
Direct sun on entire trough	6	6
Fish present in trough	0	2
Recognizable living things found in water	1	2
Additives found in water (e.g., chlorine)	0	1
Water from tap is smelly	2	0
Water from tap is cloudy	0	1
Water left iron stain	1	0
Water source is a well	6	5
Water source is same source used by humans	5	5

Table 4 shows that in six of the farms, water troughs were cleaned during the past three months prior to administering the end-of-study questionnaire. In all feedlots and in five of the dairies, wells were the source of trough water. Surface water was used to fill troughs in one dairy. In all feedlots and five dairies, the water source used to fill troughs was also used for human consumption.

Selected feeding variables for steers/heifers (age group sampled) are shown in Table 5. All study farms fed moist feeds to cattle. All dairies and none of the feedlots fed whole cottonseed. Four feedlots and no dairies fed potato waste. Three feedlots and five dairies included ionophores in rations. Two dairies and no feedlots fed brewers grains.

Table 5. Selected Feeding Variables in Study Herds

By-products Fed to Steers and Heifers	Feedlot	Dairy
Moist feeds	6	6
Whole cottonseed	0	6
Potato waste	4	0
Ionophores in ration	3	5
Brewers grains	0	2

Results of bovine cultures - Cattle positive for *E. coli* O157:H7 were identified on all farms (Table 6). The prevalence of*E. coli* O157:H7 in cattle sampled from the 12 farms ranged from 1.1% to 6.1%; for feedlots the range was 1.5% to 6.1%, and for dairies, 1.1% to 4.4%. Of 1,046 fecal samples collected in feedlots, 38 (3.6%) were positive. Of 1,097 fecal samples collected in dairy herds, 25 (2.3%) were positive.

Results of non-bovine animal cultures - Results of non-bovine culture samples are shown in Table 6. *E. coli* O157:H7 was found in the fecal samples of one horse, two dogs, and in a pooled bird droppings. It was also found in two composite fly samples. *E. coli* O157:H7 was not detected in rodent samples (275 mice and 25 other rodents), cats (n=33), or other wildlife (n=34).

Table 6. Results of *E. coli* O157:H7 Cultures from Cattle Feces, Feces of Other Animals, and Environmental Sources in 12 Pacific Northwest Cattle Operations

Sample Type	Total Samples	Number Positive	% Positive	Number of Farms with >0 Samples	Minimum Number Samples Per Farm	Maximum Number Samples Per Farm	Farms Positive for *E. coli* O157:H7[a]
Cattle - total	2143	63	2.9	12	132	194	12
Feedlot	1046	38	3.6	6	132	180	6
Dairy	1097	25	2.3	6	179	196	6
Horses	90	1	1.2	7	2	23	1
Dogs	69	2	3.2	8	1	23	1
Cats	33	0	0	3	4	24	0
Mice	275	0	0	11	3	105	0
Other Rodents[b]	25	0	0	3	1	22	0
Birds (pooled)	200	1	0.5	12	9	32	1
Flies (pooled)	60	2	3.3	11	2	10	2
Wildlife	34	0	0	6	1	14	0
Feeds	335	0	0	12	10	42	0
Trough Water	327	4	1.281	12	14	30	3
Water trough biofilm	320	6	1.98	12	13	30	5
Well Water	13	0	0	9	1	3	0

[a] *Verotoxin positive, sorbitol negative, glucuronidase negative,* E. coli *O157:H7.*
[b] *Includes 22 woodchucks, 2 rats, and 1 mole.*

Results of water trough cultures - A total of 327 trough water and 320 water trough biofilm samples were cultured. Four (1.3%) water and 6 (2.0%) biofilm samples yielded *E. coli* O157. None of the 320 matched sets of trough water and biofilm sampled simultaneously from the same troughs yielded positives. Six troughs were positive from biofilm only and four from water only.

E. coli O157:H7 positives by farm - The number of positive samples from bovine, non-bovine, and water are shown by farm in Table 7. Non-bovine animals were positive on four farms. Water troughs (water and/or biofilm) were positive on five farms. On four farms, common PFGE patterns were found in bovines and another source. On three farms bovine *E. coli* O157:H7 isolates had indistinguishable PFGE patterns as water trough biofilm isolates. On one farm, a bovine isolate could not be distinguished from a horse isolate. A dog isolate and a water trough isolate were indistinguishable on one farm. Water trough and biofilm samples were indistinguishable (same sample date but not same trough).

Table 7. Summary of Number of *E. coli* O157:H7-Positive Samples Identified from Study Farms

Codes[a]	Cattle	Horse	Dog	Fly	Bird	Trough Water	Trough Biofilm
D-1	3						1
D-2	2			1		1	1
D-3	7						
D-4	8	1					
D-5	2						
D-6	2						
F-1	7		2	1		2	2
F-2	4				1		
F-3	5					1	1
F-4	3						
F-5	8						1
F-6	11						

[a] *Numbers are arbitrary and do not relate to numbers assigned during sampling. Codes beginning with "D" designate dairies and "F" designate feedlots.*

Associations between* E. coli O157:H7 *prevalence and management variables - The distribution of *E. coli* O157:H7 cattle prevalence for farms from which *E. coli* O157:H7-positive water trough samples were found was similar to farms where *E. coli* O157:H7 was not found. Farms which reported cleaning water troughs during a three-month period (almost coincident with the study period), had similar *E. coli* O157:H7 cattle prevalence compared to farms which did not clean troughs during this period. *E. coli* O157:H7 was found from water troughs in 4 of 6 farms which did not report cleaning water and 1 of 6 farms that reported cleaning water troughs.

Discussion

This study agrees with results of previous studies that found *E. coli* O157:H7 to be widespread in cattle operations. Indeed, the failure to find one negative herd supports the conclusion that the organism is found everywhere. This has important implications for choice of control method. Eradication and other methods based on traceback of positive cattle to farms of origin would not be viable alternatives for such an ubiquitous agent.

The results of the present study support the conclusion that *E. coli* O157:H7 is not a single-host organism. It provides clear evidence that *E. coli* O157:H7 can colonize multiple species on a farm. In addition to considerable data on its ability to colonize cattle and humans, the present study indicates that *E. coli* O157:H7 can colonize several other species as well, including horses, dogs, and birds. While it can be argued that these other species are only transiently colonized rather than being long-term hosts for *E. coli* O157:H7, the same argument can be made for cattle. It remains for future studies to determine which of these species, if any, is the reservoir.

Notably, *E. coli* O157:H7 was not found among the 300 rodent samples in this study. Rodents have come under justifiable scrutiny due to their well described role for certain Salmonella serotypes, because they are present on most farms, and because they are in intimate contact with livestock feeds. Approximately 1/3 (n=105) of the rodent samples in the present study came from one farm which somewhat reduces the power associated with a sample size of 300. Nevertheless, rodents were captured and cultured from 11 cattle farms in which *E. coli* O157:H7 was found to be endemic. If rodents were the major reservoir for *E. coli* O157:H7, it seems highly unlikely that it would not have been detected in the present study.

The most common non-bovine source of *E. coli* O157:H7 was water troughs. Clearly, water troughs can represent a potential vehicle by which the agent could be spread from one animal to another. It also seems possible that water

troughs may represent a long-term source of *E. coli* O157:H7. That biofilm samples were more frequently positive than water itself suggests that *E. coli* O157:H7 may be sequestered in this complex ecosystem which consists of a variety of algae, bacteria, protozoa, and, in some cases, arthropods and nematodes. The ability of *E. coli* O157:H7 to survive for long periods and even multiply in aquatic sediments has been described.

Future Work

In a previous pilot study, 80% of the feeds sampled were contaminated with *E. coli* O157:H7. Previous work also indicated that corn silage may be a risk factor for high prevalence of *E. coli* O157:H7 within dairy herds.

Isolation of *E. coli* O157:H7 from flies and birds in this study indicates that the potential also exists for these wildlife to be sources of contamination of feed in storage. Even though *E. coli* O157:H7 was not isolated from the feed samples in this study, systematic statistical sampling of the feeds was not performed. A better study design to evaluate feeds adequately needs to be conducted. Between exposure to contaminated water and potentially contaminated feeds, these factors may explain the intermittent shedding pattern of *E. coli* O157:H7 by cattle which, again, appears to be only transient carriers of this organism.

Risk Factors for Verotoxin-positive *Escherichia coli* O157:H7 in Pacific Northwest Dairy Herds

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Abstract

To determine the risk factors associated with verotoxin-positive *Escherichia coli* O157:H7 in Pacific Northwest dairy herds, a total of 36 herds in Washington, Oregon, and Idaho, were selected based on the following criteria: (a) at least 60 post-weaned heifers will be available for sampling throughout the six-month study period, (b) owners are willing to provide management information, and (c) owners will allow sampling over the length of the study period. Sample collection ran from July 5 to December 20, 1994. Of the 12,664 fecal samples collected, 179 (1.41%) were culture-positive for *E. coli* O157:H7. *E. coli* O157:H7 was detected in 27 of the 36 herds (75%). Within-herd prevalence ranged from 0% to 5.5%, with a strong clustering toward the lower end of this range. Thirteen herds had heifers exposed to manure-applied pasture at some time during the study. These exposed herds had a distribution of *E. coli* O157:H7 prevalence similar to two types of herds in the study: (1) herds which had heifers grazed on pasture where manure had not been applied during the growing season, and (2) herds whose heifers were maintained in drylots. Several nutritional variables were associated with *E. coli* O157:H7 prevalence or presence. Seven (3.4%) of the 205 cull cows from the study herds sampled at the farm of origin tested positive. Four (3.9%) of the 103 cull cows from 15 study herds sampled at slaughter were

positive. Of the 89 cull cows sampled both at the farm and at slaughter, 2 (2.2%) cows at both locations, 2 (3.3%) only on the farm, and 2 (2.2%) only at the slaughter plants, were positive. Of the 89 cull cows tracked from farm to slaughter, 7 (7.9%) were positive in at least one location. Findings of this study must be interpreted with caution due to its limited sample size; however, the findings suggest that *E. coli* O157:H7 prevalence may increase several-fold in cattle being held or transported for slaughter. This stage of production may represent an important critical control point for *E. coli* O157:H7.

Purpose

E. coli O157:H7 was first recognized as a human pathogen in 1982 although evidence exists that a pathogenic relationship existed prior to that date. Although other sources have not been ruled out, cattle have been implicated as a major source of human exposure. However, it has not been established beyond doubt that cattle are a reservoir rather than incidental hosts.

The purposes of this study were to:

1. Provide an estimate of herd prevalence of *E. coli* O157:H7 based on intensive sampling in 36 Pacific Northwest dairy herds.

2. Estimate the level of diversity of within-herd *E. coli* O157:H7 prevalence among herds and the level of temporal clustering of *E. coli* O157:H7 shedding.

3. Determine if an association exists between heifer environment - notably grazing on land to which manure has been applied - and within-herd prevalence of *E. coli* O157:H7.

4. Screen a wide variety of management variables for possible association with *E. coli* O157:H7 prevalence.

5. Determine the prevalence of *E. coli* O157:H7 in cull dairy cows on the farm and after arrival at the slaughter plant.

Materials and Methods

Study herds - Twelve herds each (36 total) from Washington, Oregon, and Idaho were selected based on the following criteria: (a) at least 60 post-weaned heifers would be available for sampling throughout the next six months, (b) owner willingness to provide management information, and (c) owner willingness to allow sampling over six months. Four herds were selected in each of the three states that observed the following herd management practice: (1) grazed heifers on pastures where manure has been applied (M), (2) grazed

heifers on pasture where manure had not been applied in the current growing season (P), and (3) maintained heifers in drylots only (D). In each state, section veterinarians and animal health technicians (either state or federal) were assigned the specific type of farms to locate. The section veterinarian selected and sampled the participant farms.

Sample collection - Sample collection ran from July 5 to December 20, 1994. In each herd, approximately 60 fecal samples from post-weaned heifers were collected monthly. Two herds had only five monthly collections because the herd had been sold or were inaccessible. Fresh fecal pats were sampled using cotton-tipped swabs, which were placed in tryptic soy broth containing 40μg/ml vancomycin and 50ng/ml cefixime. The samples were refrigerated and shipped overnight to the laboratory.

E. coli O157:H7 *assay* - After arrival at the laboratory, samples were briefly agitated, incubated for 24 hours at 37°C and diluted to 10^{-4}. Dilutions were plated onto sorbitol MacConkey containing 50ng/ml cefixime and 2.5 μg/ml potassium tellurite and then incubated overnight at 30°C. Up to 10 sorbitol non-fermenting colonies (if that many were present) were selected from each sorbitol MacConkey plate and tested for lactose fermentation using MacConkey agar. Sorbitol non-fermenting, lactose fermenting colonies were tested for glucuronidase activity using a 4-methlyumbelliferyl-beta-D-glucuronide (MUG) assay performed in 96 well plates. MUG negative colonies were tested for O157 antigen by slide agglutination. Positive colonies were assayed for verotoxin genes using a polymerase chain reaction with primers specific for VT-1 and VT-2.

Collection of farm data - During the first visit to each farm, a questionnaire was completed covering calf, heifer, and cow management variables. On each subsequent visit, an update questionnaire was completed for each herd.

Manure slurry testing - Fecal coliform counts were measured in two samples of stored manure collected from each of the 12 herds in which manure was applied to grazing land during two consecutive monthly visits. A standard membrane filter technique was used.

Positive herd - A herd is considered positive if *E. coli* O157:H7 is found in the herd one or more times over the sampling period of six months.

Status at time of sampling - The location of heifers at the time of sampling was recorded. The information recorded for M herds included whether or not manure had been applied to the pasture where heifers grazed in the past 30 days.

Statistical Methods

The Kruskal-Wallis test was used to compute index P-values for differences in distributions of *E. coli* O157:H7 prevalence between management groups. Log linear analysis was used to test significance of joint effects of two management variables on herd detection status of *E. coli* O157:H7.

Results

Descriptive Statistics and Associations

A total of 12,664 fecal samples were collected from heifers during 214 visits to the 36 study farms. Of these samples, 179 (1.41%) were culture-positive for *E. coli* O157:H7.

E. coli O157:H7 was detected in 27 of the 36 herds (75%). Within-herd prevalence ranged from 0% to 5.5% with a strong clustering toward the lower end of this range (Figure 1). The highest *E. coli* O157:H7 prevalence noted in any herd on any single sampling occasion was 26.7% (19 of 62 samples during the third sampling period). Of the 214 sampling visits, 147 (68.9%) did not yield positive samples. A moderate correlation was noted among within-herd prevalence on successive visits indicating a tendency for herds to maintain relatively low or high prevalence status.

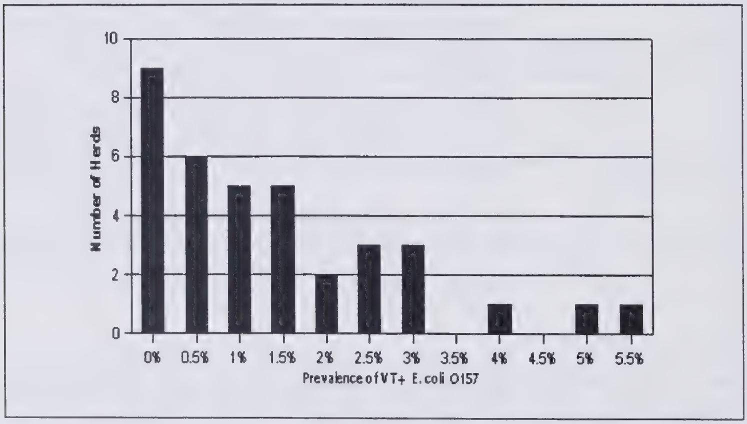

Figure 1. Frequency distribution of E. coli O157:H7 within-herd prevalence among heifers in 36 dairy herds in the Pacific Northwest.

E. coli O157:H7 positive animals were detected during each of the sampling periods. The highest prevalence of 2.0% was observed during the third sampling period (August 29 to September 20) and the lowest (0.5%) prevalence during the final sampling period (November 28 to December 20).

Study herds varied greatly in size. In general, no correlation was found between herd census variables and prevalence of *E. coli* O157:H7; however, a moderate correlation was observed between *E. coli* O157:H7 and number of bulls and steers.

Environmental Variables

There were 13 herds whose heifers were exposed to manure-applied pasture (M) some time during the study. These herds had a distribution of *E. coli* O157:H7 prevalence similar to that of herds which had heifers grazed on manure-free pasture during the growing season (P) and herds whose heifers were maintained in drylots (D).

A slightly higher proportion of M herds (85%) had at least one positive sample when compared to D (82%) or P (66%) herds. Yet, median within-herd prevalence was highest for D herds (1.66%) compared to P herds (0.42%) and M herds at 0.83% (P=.49). In seven herds in which a 1-day waiting period after manure application prior to grazing was observed, the median prevalence of *E. coli* O157:H7 was 0.83% compared to 1.09% in other herds. In five herds where heifers grazed on pasture in which manure was applied and had less than 30 days of manure storage, the median prevalence of *E. coli* O157:H7 was 0.83% compared to 1.09% in other herds.

When *E. coli* O157:H7 prevalence was computed based on heifer environment at the time samples were collected, heifers in drylots tended to have a higher prevalence throughout the sampling period than did pastured heifers. The prevalence of all three environment groups (drylot, pastured/manure, pastured/no manure) declined sharply during the final sampling period (November 28 - December 20).

Producers were questioned on a monthly basis about application dates of manure to pasture in which heifers grazed. A total of 1,057 samples were collected from animals grazed on pasture to which manure had been applied in the previous 30 days. Seventeen of the 1,057 samples were positive for *E. coli* O157:H7 (1.6%) compared to 127 of 9,478 (1.3%) in samples collected from heifers that grazed on pastures to which manure had not been applied in the last 30 days (P=.48).

The median prevalence of *E. coli* O157:H7 in 17 herds which applied manure to grain or truck crops was 0.55% compared to 1.66% in other herds. Among 26 herds which applied manure to forage crops, the median prevalence of *E. coli* O157:H7 was 1.37% compared to 0.00% among herds which did not (P=.06). However, the 10 herds which applied manure to forage crops and observed a waiting period of less than 30 days until harvest had a median prevalence of 0.56% compared to 1.37% among other herds (P=.45) which never applied manure to forage or maintained a waiting period of 30 days prior to harvest.

Samples from manure storage sites had generally high numbers of fecal coliforms. Of the 12 herds that applied manure to heifer grazing land, 8 had average concentrations of fecal coliforms 10,000 CFU/ml.

Feeding and Weaning Variables

Thirteen calf management variables were evaluated for possible association with *E. coli* O157:H7. The variables focused on the areas of weaning and grouping strategies, age of onset of calf starter and forage feeding, protein content of forage and calf starter, and several specific starter ingredients which were found to vary among study herds. Weaning method and protein content of calf starter were associated with *E. coli* O157:H7 prevalence. Among 17 farms which abruptly weaned calves, the median *E. coli* O157:H7 prevalence was 1.67%. This compared to median prevalence of 0.82% in six herds which gradually weaned calves by reducing the amount of feed, 0.0% in three herds which gradually weaned by diluting milk, and 0.83% in nine herds which gradually weaned calves by increasing the interval between feedings (P=.07). Of the 17 farms which fed calf starter containing more than 16% protein concentration, the median prevalence was 1.37% compared to 0.28% for farms feeding a lower protein level starter (P=.13).

Sixteen heifer management variables related to the period between weaning and first calving were examined for possible association with *E. coli* O157:H7. These management variables focused on the areas of feed additives, amount of grain fed, use of corn silage, and use of several by-product feeds. The strongest association was observed for corn silage feeding. Although few herds fed corn silage to heifers under 12 months of age, a higher prevalence of *E. coli* O157:H7 was noted for all age groups in herds fed with corn silage. When corn silage was fed to 12-18 month-old heifers in 13 herds, the median prevalence of *E. coli* O157:H7 was 1.73%, compared to 0.28% in the remaining herds (P=.03). In one-month old pre-calving groups in 17 herds fed with corn silage, the median prevalence of *E. coli* O157:H7 was 1.73% compared to 0.28% in the remaining herds (P=.01).

A more tentative association was observed between *E. coli* O157:H7 prevalence and the use of feed additives. For herds using monensin, lasalocid, and decoquinate, the prevalence was higher than among herds that did not use these substances. The association appeared to be strongest for monensin (median prevalence = 1.73% in herds using monensin compared to 0.69% in herds not using the additive, $P=.10$); however only six herds used monensin. The median prevalence in 27 herds which used at least one of the three feed additives was 1.66% compared to 0.27% in the remaining nine herds ($P=.06$).

The joint effects of the feeding of corn silage and ionophores (monensin or lasalocid) were examined graphically and with log-linear analysis. Controlling for ionophore use, corn silage feeding was significantly ($P>.01$) associated with *E. coli* O157:H7 herd status (positive or negative). Controlling for corn silage use, supplementation with ionophores was significantly associated with *E. coli* O157:H7 herd status ($P=.07$). No significant interaction was detected ($P=.48$). It should be noted that six of the nine herds in which *E. coli* O157:H7 was not detected belong to the 10 herds in which neither corn silage nor ionophores were fed.

Of by-products fed to heifers between weaning and first calving, an association was noted with *E. coli* O157:H7 only in the case of grain screens. Though only eight herds used this product, the median prevalence in these herds was 2.10% compared to 0.96% in the remaining herds ($P=.14$).

Seven variables related to adult cow management and feeding were examined for possible association with *E. coli* O157:H7 prevalence in heifer samples. Among six by-products fed to cows in some of the study herds, animal by-products was the only feed which appeared to be associated with the prevalence of *E. coli* O157:H7. *E. coli* O157:H7 was detected in all of the 11 herds that used animal by-products as feed with a median prevalence of 1.67%. This compared to 16 of 25 positive herds, and a median prevalence of 0.55% for those herds that did not feed animal by-products ($P=.09$).

Entry of outside cattle into a herd during the study period was not associated with a higher prevalence of *E. coli* O157:H7. Among 10 herds which admitted new animals, the median prevalence was 1.25% compared to 1.09% among herds which did not ($P=.85$).

Cull Cow Sampling

A total of 205 cull cows from 19 of the study herds were sampled at the farm of origin. Seven (3.4%) of the 205 cull cows tested positive. Of 103 cull cows from 15 study herds sampled at slaughter, four (3.9%) were positive. Of 89

cull cows sampled both at the farm and at slaughter, two (2.2%) were positive in both locations, three (3.3%) only on the farm, and two (2.2%) only at the slaughter plant. Of the 89 cull cows tracked from farm to slaughter, seven (7.9%) were positive in at least one location. All *E. coli* O157:H7 positive cull cows were observed in the months of July-October (sampling continued into December). All positives were from farms which had heifers identified as *E. coli* O157:H7 positive. An additional 96 cull cows from farms other than the study herds were sampled at slaughter plants in December; none of them were positive for *E. coli* O157:H7.

Discussion

The results of this study suggest a herd prevalence of *E. coli* 0157:H7 in the Pacific Northwest dairy herds of at least 75%. It is possible that additional sampling may result in the detection of *E. coli* O157:H7 on some of the nine, perhaps all, farms where it was not detected in six sampling visits. These findings are in contrast to herd prevalence estimates of <10% made in previous studies. The difference may be attributed, perhaps, to the superior sampling design of the present study.

Strong evidence of temporal clustering is demonstrated by the fact that no positive samples were found in 68.9% of sampling visits, even though 75% of herds were eventually found to be positive. It is noteworthy that more intensive follow-up sampling revealed *E. coli* O157 in many of the herds which tested negative in both the National Dairy Heifer Evaluation Project (NDHEP) and University of Washington studies.

The weakness of the present study, if any, is that only a relatively small number of herds (n=36) were sampled and herds were not truly randomly selected. However, the intensive sampling employed in each herd could not have been extended to a larger number of herds without substantially increasing resources beyond those necessary for the collection and assay of the 12,664 fecal samples in the present study. Also, even though selection was not statistically random, there is no *a priori* evidence that the herds selected for this study would have a greater or lesser *E. coli* O157:H7 prevalence than other Pacific Northwest herds.

The main implication of the near ubiquitous distribution of *E. coli* O157:H7 relates to currently popular proposals for traceback programs. Presumably, a traceback program would work only if a small percent of herds represented the source for human foodborne exposure to *E. coli* O157:H7. Under this assumption, a traceback program would provide an efficient means of identifying and eliminating the small fraction of herds endemic for *E. coli*

O157:H7. The finding in this study that *E. coli* O157:H7 is present in most herds seems to rule out this strategy.

One competing alternative to traceback programs as a pre-harvest strategy involves the identification of farm management factors that modulate the occurrence of *E. coli* O157:H7. If such factors exist, they could be exploited in an effort to reduce the overall prevalence of the agent in cattle populations. The wide variation of within-herd prevalence in the present study is solid evidence for differences in *E. coli* O157:H7 ecology among herds (see Figure 1) and, by inference, for modulating factors. The prevalence correlations observed during the six sampling periods indicate that the tendency of a herd to have relatively low or high prevalence is a relatively stable phenomenon. This is the expected pattern for differences in prevalence produced by the action of management variables as modulating factors on the ecology of *E. coli* O157:H7.

As in previous studies, this study found several nutritional variables associated with the prevalence or presence of *E. coli* O157:H7. Based on these associations and on the biological plausibility of an effect of diet on intestinal floral ecology, it seems very likely that some dietary influences on *E. coli* O157:H7 do exist. The details of this relationship, however, cannot be properly characterized in studies such as this one. This study and the other broad etiologic studies on the subject have been in substantial disagreement as to which particular nutritional influences are critical. For example, two previous studies saw a relationship between feeding of whole cottonseed and the presence of *E. coli* O157:H7 in dairy herds. This study, however, did not discover this association. One of the previous studies saw an association between *E. coli* O157:H7 detection and ionophore use as did the present study, but another study examining this relationship failed to find any association. This is the first, and heretofore only, study which observed a relationship between corn silage feeding and the presence or prevalence of *E. coli* O157 in heifers.

Definitively identifying the critical nutritional factors involved in modulating *E. coli* O157:H7 ecology will require controlled experiments. Ideally, intervening variables, such as measures of gastro-intestinal floral stability, would feature prominently in such studies since this would be a much more satisfactory dependent variable than *E. coli* O157:H7 prevalence alone. Identifying an association of the putative modulating factors with intervening variables would also give much greater confidence in the causal inferences made from statistical associations.

Although the cull cow sampling described in the present study was limited, the results suggest that this may be a critical component of human foodborne exposure. Among the 89 cows followed from farm to slaughter, 7.9% were positive on the farm or at the slaughter plant. This is a much higher prevalence than previously reported for adult cattle, and higher even than that observed in heifers. Heifers have been reported, in several studies, to be the highest prevalence group. Though the findings of the present study must be interpreted with caution due to its limited sample size, the findings suggest that *E. coli* O157:H7 prevalence may increase by several-fold in cattle being held or transported for slaughter. This stage of production may represent an important critical control point for *E. coli* O157:H7.

Prevalence and Clonal Nature of *Escherichia coli* O157:H7 on Dairy Farms in Wisconsin

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Abstract

A survey was conducted between March and October of 1994 to determine the prevalence and identify the source of serotype O157:H7 isolates of *Escherichia coli* in Wisconsin dairy herds. A stratified sample of 400 farms was identified and 70 farms with weaned calves less than four months of age were tested. In the prevalence study, 5 of 70 farms (herd prevalence of 7.1%) and fecal samples from 10 of 560 calves (animal prevalence of 1.8%) tested positive for serotype O157:H7. In the case-control study, the five *E. coli* O157:H7-positive farms (case farms) and seven *E. coli* O157:H7-negative (control farms) identified in the prevalence study were again visited. Of an additional 517 fecal samples from cattle of various age groups, 19 total animals from 4 of 5 case herds (15 animals) and 2 of 7 control herds (4 animals) tested positive. Epidemiological data suggested that horizontal transmission was an important means of *E. coli* O157:H7 dissemination on the farm. Of 302 environmental samples, 2 animal drinking water samples from 1 control farm and 1 water tank sample from a case farm contained *E. coli* O157:H7.

Analyses by the pulsed-field gel electrophoresis technique of contour clamped homogeneous electric fields electrophoresis using XbaI revealed that isolates from the same farm displayed identical or highly similar restriction endonuclease digestion profiles (REDP), whereas isolates from different farms typically displayed different REDP. However, more than one REDP was usually observed for a given herd over the seven-month sampling period. Analyses of multiple isolates from a single animal revealed that some animals harbored O157:H7 strains with different REDP, although the REDP of isolates from the same fecal sample were highly similar. Collectively, the 160 bovine isolates (from 29 animals) and 3 water isolates displayed 20 distinct XbaI REDP. Our data revealed the presence of several clonal types of serotype *E. coli* O157:H7 isolates in Wisconsin and indicated there was probably more than one source of this pathogen on dairy farms. However, animal drinking water was identified as a potential reservoir for *E. coli* O157:H7 on a farm.

Materials and Methods

Prevalence and case-control studies. - A stratified sample of 400 dairy farms in Wisconsin was identified. The farmers were contacted by letter and phone, and 70 agreed to participate in the study.

Case herds were identified in the prevalence study as having one or more weaned calves that tested positive for *E. coli* O157:H7. Control herds tested negative for *E. coli* O157:H7 in the prevalence study were matched by herd size (i.e., stratum) and geographic location to case herds.

Sample collection and storage. - Samples were collected from March through October 1994. In the prevalence study, only weaned calves less than four months in age were tested. All bovine fecal samples (about 30g) were obtained by digital rectal retrieval. Fecal samples from cats and dogs were collected using a rectal loop, and pig and rabbit feces were collected from cages or pens housing individual animals. Racoon fecal sample was obtained from a barn floor. All fecal samples were transferred to sterile, screw-cap tubes containing 7.5 ml of Bacto Transport Medium without agar (Difco Laboratories, Detroit, MI) and shaken. Environmental, feed, water, and non-fecal animal samples were collected aseptically and transferred to sterile containers (i.e., Whirlpack bags or specimen cups). All samples were then placed in coolers containing cold packs and shipped overnight to the Food Research Institute (Madison, WI) for testing. If necessary, the samples were refrigerated prior to express delivery, but all samples were tested within 48 hours of collection.

After portions of fecal samples were removed for microbiological testing, the remainder of the sample was mixed 1:1 with 2x Trypticase soy broth (BBL Microbiological Systems, Cockeysville, MD) containing 20% glycerol and stored at -20°C.

Microbiological analyses. - A 10 g portion of a sample was added to a flask containing 90 ml of modified EC broth plus novobiocin (Sigma Chemical Co., St. Louis, MO) with final concentration of 20 μg/ml and incubated at 37°C with shaking (100 rpm) for 18 to 24 hours. When testing water samples, 100-ml volumes were added to 100 ml of 2x modified EC broth and incubated as described for the other samples. Next, samples were serially diluted in 0.1% Bacto peptone (Difco), and 0.1-ml of the 10^{-5} and 10^{-6} dilutions were spread plated onto duplicate plates of MacConkey Sorbitol Agar (MSA, Difco). In the case-control study, MSA supplemented with cefixime (50 μg/L; Lederle Labs, Pearl River, NY) and potassium tellurite (25 mg/L; Sigma) [MSA + (25)] was used in addition to MSA to further enhance detection of *E. coli* O157:H7. Plates were incubated overnight at 42°C and examined for the presence of sorbitol-negative (i.e., white) colonies. A maximum of 15 sorbitol-negative colonies per positive sample were tested for the 0157 antigen by agglutination

(Oxoid, Basingstoke, England). Colonies that agglutinated were streaked onto MSA, incubated overnight, and retested for the 0157 antigen. Agglutination-positive isolates were then transferred to brain heart infusion (Difco) agar slants until biochemical and serological tests were conducted.

Sorbitol-negative and 0157-positive colonies were biochemically confirmed as *E. coli* using an API 20E biochemical test strip (bioMerieux Vitek, Inc., Hazelwood, MO). Isolates were also tested for ß-glucuronidase activity using 4-methylumbelliferyl-ß-d-glucuronide (Sigma)(20) and for the presence of the H7 antigen using antiserum as described by the manufacturer (Difco). Confirmed colonies from each sample (maximum of 12) were stored in nutrient broth (Difco) containing 10% glycerol at -70°C for further analyses.

Genomic typing. - The pulsed-field gel electrophoresis (PFGE) technique of contour clamped homogeneous electric fields (CHEF) electrophoresis was used for genomic typing of *E. coli* 0157:H7 isolates (12, 16). Genomic DNAs were digested in agarose plugs using XbaI (Promega Corp., Madison, WI) as recommended by the manufacturer. The resulting fragments were resolved by CHEF/PFGE using a CHEF-DRII apparatus (Bio-Rad Laboratories, Richmond, CA) at 200V for 21 h at 21°C and switch times ramped from one to 40 seconds. Lamba concatamers (New England Biolabs, Inc., Beverly, MA) were used as a DNA size standard.

Detection of Shiga-like toxin genes. - Two 20-base pair oligonucleotide probes (13) were purchased (National Biosciences, Plymouth, MN) and used to detect the presence of Shiga-like toxin (SLT) I and II genes (16). The probes were labeled with digoxigenin and hybridization was detected following procedures described by the manufacturer (Boehringer Mannheim, Indianapolis, IN).

Data management and calculation of similarity indices. - The presence or absence of macrorestriction fragments for each strain was transcribed into binary scores for analysis by ELBAMAP software, and the number of shared fragments between REDP was used to calculate the Dice similarity index as described by Brosch, et al.

Results

Prevalence study. - The survey of 70 Wisconsin farms revealed that five of the 70 farms (herd prevalence 7.1%) and 10 of 560 weaned calves (animal prevalence of 1.8%) tested positive for *E. coli* O157:H7.

Case-control study. - The five farms that tested positive for *E. coli* O157:H7 in the prevalence study all belonged to stratum ranging from 134 to 274 cattle.

The seven control farms that tested negative for *E. coli* O157:H7 in the prevalence study were matched to the five case farms by stratum and geographic location. On subsequent visits to each of the five case and seven control farms, an additional 517 fecal samples from cattle of various age groups were tested. Fifteen animals from four of five case herds and four animals from two of the seven control herds tested positive for *E. coli* O157:H7.

Further analyses of data from *E. coli* O157:H7-positive herds found that positive animals shared water, inhabited the same barn or pen, occupied a pen previously containing a positive animal, or were located in an area in close proximity to a positive barn or pen.

In addition to fecal samples, a total of 302 environmental, feed, water, and non-fecal animal samples were examined for *E. coli* O157:H7 during the case-control study.

Of these 302 samples, only three water samples (3 of 101) tested positive for *E. coli* O157:H7. Two of the positive animal drinking water samples were from a tank on a control farm and one sample was from a water trough on a case farm.

A single isolate from each of the 10 positive calves identified in the prevalence study was examined by CHEF/PFGE. Four distinct REDP were identified among the 10 isolates examined. From these prefatory findings, it appeared that each farm had a distinct REDP, with farms A and H containing an identical REDP (type 33) despite being located approximately 75 miles apart.

Genomic typing of the *E. coli* O157:H7 isolates obtained from the case-control study demonstrated multiple REDP types within a herd and established that REDP present in a herd can change over time.

To determine if different *E. coli* O157:H7 were present in a given animal, a maximum of 12 colonies from a single fecal sample were analyzed by CHEF/PFGE.

Genomic typing of these 160 isolates revealed that an animal may harbor *E. coli* O157:H7 that displays a different REDP. For example, seven of the 29 positive animals (24%) harbored *E. coli* O157:H7 with a different REDP, and two animals carried three different REDP.

A total of 19 REDP were isolated from dairy cattle in Wisconsin and one additional REDP was found in an animal drinking water sample.

Presence of SLT genes. - At least one isolate representing each of the 20 REDP identified was examined using digoxigenin-labeled oligonucleotide probes to SLT I and II. Thirteen strains were positive for both SLT I and II, while one was positive of only SLT I and six were positive for only SLT II. Isolates with the same REDP had the same toxin profile, but isolates with the same toxin profile had varying REDP. It is noteworthy that when multiple REDP types were present in a single animal, the strains had the same toxin profile. These data indicate that although toxin typing can provide insight on the virulence potential of isolates, genomic fingerprinting is a more discriminating typing method.

Discussion

Results from our survey in Wisconsin found 7.1% of dairy herds positive for *E. coli* O157:H7. Although the herd prevalence in Wisconsin was similar to findings in the state of Washington, it likely underestimates the actual prevalence because the number of calves tested on each farm was based on the results of Garber et al. which reported that 5% of weaned calves shed *E. coli* O157:H7 and only approximately 1.8% of weaned calves tested positive in this study.

On subsequent visits to previously positive (case) and negative (control) farms, four of the five (80%) case and two of seven (29%) control farms tested positive for *E. coli* O157:H7. Previous studies have also reported changes in the *E. coli* O157:H7 status of farms.

Results from our case-control study also revealed that *E. coli* O157:H7-positive animals shared the same barn, pen, or water, occupied a pen that previously contained a positive animal, or were located in areas in close proximity to a positive barn or pen. Grouping of preweaned calves was also associated with the *E. coli* O157:H7 herd status in another study.

These data suggest that direct transmission among animals and indirect transmission through contact with areas previously contaminated by animals shedding *E. coli* O157:H7 are important factors in disseminating this pathogen in a herd. Likewise, the detection of *E. coli* O157:H7 in a water tank/trough and the failure to detect it in a limited number of other environmental samples indicate that contaminated water may also be an important reservoir for this pathogen on farms.

A total of 20 REDP were identified among the 160 *E. coli* O157:H7 isolates recovered from fecal samples as well as three water samples tested. The REDP were generally similar and unique to each farm.

Results of this study suggest that there is little farm-to-farm transmission and there is likelihood that there is more than one source of *E. coli* O157:H7 in a farm.

Water was the only non-fecal sample that tested positive for *E. coli* O157:H7. Although water has been implicated in human outbreaks, this study demonstrated that water may be an important source of *E. coli* O157:H7 in farms.

Future Work

Phase III of this project will follow three positive farms and one negative farm as designated from the Case/Control Study for1-1/2 years. A cohort group of 15 or more calves starting from birth and through the first 18 months of life (possibly beyond) will be sampled. Sampling of calves' feces will be done weekly, except during times when management changes occur (stresses such as weaning, dehorning, grouping, diet changes, etc.) During these times of stress, more intense daily fecal sampling of the cohort group and any in contact animals will be done. In the event of positive isolations, the study will be expanded to include environmental variables. Data will be compiled concerning all life events and management occurrences and will becorrelated with shedding of *E. coli* O157:H7. Questionnaires will be completed by the producers and extensive records will be kept on the cohort group.

Sampling of possible vectors or carriers of *E. coli* O157:H7 on the farm will be performed. Of particular interest and subsequently target organisms are mice, rats, birds, rabbits, deer, and flies. Surrounding water sources are also being sampled, because water has been identified as one of the primary transmission modes for *E. coli* O157:H7. Adult animals on the farm at times of stress (especially cows before and after parturition and during any type of sickness or injury, before shipment to slaughter and at slaughter if possible) will be sampled.

All fecal isolates will be fingerprinted using Pulse-Field Gel Electrophoresis (PFGE) and then stored in an isolate bank for future reference.

New York Cull Cow Study on *Escherichia coli* O157:H7

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Abstract

Fecal samples were obtained from 1,602 cull dairy cows prior to slaughter. The cows originated from the State of New York. Fecal samples were also obtained just prior to slaughter from 67 non-ambulatory suspect cows retained in the suspect holding pens. All samples were cultured for *Escherichia coli* O157:H7 using the direct plating technique. Results show the following: 14 of 1,602 (0.8%) ambulatory, 2 of 66 (3%) non-ambulatory, and 16 of 1,668 (1.0%) total number of cows sampled were positive.

Purpose

This study screened for *E. coli* O157:H7 (and other potential enteric infections) in dairy cattle immediately before they were slaughtered. The study also compared marketing conditions, cow condition, and cow quality.

Materials and Methods

Sampling of Cull Cows

Fecal samples were obtained from 1,602 cull dairy cows prior to slaughter. The cows originated from the State of New York. Cows were processed in a ranger chute transported and set up on premises. Fecal samples were also obtained just prior to slaughter from 67 non-ambulatory suspect cows retained in the suspect holding pens.

Data Collection

Data were collected on the day of sampling. Data obtained at the packing plant included most recent sale of dealer, trucking time and conditions from sale, number of sale stops per truck load, all sale tag and farm identification. Number of sales and time since leaving the farm prior to first sale were determined and analyzed for a random sample of all cows tested.

Additional data collected on each cow to evaluate any relationship to *E. coli* O157:H7 or carcass grade included Body Condition Score (BCS), hide cleanliness score, ambulatory score, manure consistency, and obvious lesions.

Results

All samples were cultured for *E. coli* O157:H7 using the direct plating technique. Results show the following: 14 of 1,602 (0.9%) ambulatory, 2 of 66 (3%) non-ambulatory, and 16 of 1,668 (0.8%) total number of cows sampled were positive.

Direct plating procedures on Sorbital MacConkey agar were used to culture *E. coli* O157:H7 and evaluate the predicted prevalence in cull dairy cows. Although direct plating is the standard isolation procedure for *E. coli* O157:H7, it is not the most sensitive detection method. Enriched sensitivity procedures have been developed and published most recently by Dale Hancock (J. Clinical Micro. Oct 1995, p. 2616-2619). This procedure was not used due to significantly increased labor and media costs. All samples were frozen at -70°C for further analysis.

Further Work

Data will be collected from other sources in order to determine the marketing routes and amount of time cows spend in the marketing system. An additional 600 animals will be sampled at the packing plant in spring, summer, and winter of 1996. The same data will be collected and an enrichment dilution culture method will be used on these samples. Concurrently, a sensitive multiplex PCR method to detect *E. coli* O157:H7 is being developed. It is intended as a less labor intensive screening test. Enhanced bacteriologic culture will be performed on all PCR-positive samples. A comparison on a random selection of the 1669 samples will be performed to evaluate differences in direct plating vs. the enrichment and dilution techniques.

Sensitivity and specificity of this new PCR method will be evaluated and compared to the standard and enriched plating techniques. Data are being entered into a database for analysis. This will provide preliminary findings upon which the Enhanced Pathogen Projects (EPP) will be based. The first EPP will be a case-control study of the positives and randomly selected controls.

Preliminary Report:

Prevalence of Verotoxic *Escherichia coli* O157:H7 in Lambs at Slaughter

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Abstract

This study determined the prevalence of verotoxic *Escherichia coli* O157:H7 in market lambs prior to slaughter. Upon arrival of the animals at a midwestern slaughter establishment, fecal samples were obtained via rectal extraction. Of the 882 samples, 160 were swabs and 722 were pellets. Overall, 8 of 882 (0.9%) lambs sampled were found positive for *E. coli* O157:H7, 1 of the swabs, 6 of the pellets, and 1 of the 90 10-gm samples. The 10-gm sample found positive was not identified as positive through culturing a single fecal pellet.

Introduction

Microorganisms gain access into or onto meat from many different sources during the growing, processing, and handling of meat animals. The identification of *E. coli* O157:H7 as a particularly pathogenic bacterial agent to humans has initiated research into its ecology and prevalence in a variety of environments. To date, the focus of attention has been on the beef and dairy industries. Previous studies have estimated animal prevalence to range be-

tween 0 and 2.2% and herd prevalence to range between 1.8 and 100% on different types of cattle operations.

Although ground beef has been the vehicle implicated in most human cases of *E. coli* O157:H7, it is not clear that cattle are the only reservoir for the agent. *E. coli* O157:H7 subtypes sometimes appear suddenly in cattle herds and then disappear, which suggests the existence of other host species. Sheep is an important candidate species to be considered for this role for at least two reasons: (1) sheep can be experimentally colonized with *E. coli* O157:H7, and (2) the agent has been found naturally colonizing at least one Idaho sheep flock. That no *E. coli* O157:H7 outbreaks have been traced to sheep could be explained by the low consumption of lamb in the U.S. compared to beef. Investigators have identified the presence of *E. coli* O157:H7 in retail sheep products. A finding of widespread colonization of sheep with *E. coli* O157:H7 would have important implications for decisions related to control.

The purpose of this study was to determine the prevalence of *E. coli* O157:H7 in market lambs just prior to slaughter and to identify factors that may be associated with higher or lower prevalence.

Materials and Methods

Lambs were placed in pens bedded with straw upon arrival at a midwestern slaughter establishment. Fecal samples were obtained by rectal extraction. Depending on lot size, 30 to 60 animals were sampled from each lot. Single fecal pellets were collected from sheep which had pelleted feces, otherwise a swab was collected. Samples were collected and shipped in trypticase soy broth (TSB) to which had been added 40 mg/ml vancomycin and 50 ng/ml cefixime. Without addition of other reagents, these enrichment solutions were incubated at 37°C within 24 hours of arrival at the laboratory. Dilutions to 10^{-4} were prepared in TSB. Three hundred µl of the 10^{-3} and 10^{-4} dilutions were plated onto 150 mm sorbitol MacConkey plates containing 50 ng/ml cefixime and 2.5 mg/ml tellurite (SMACtc) using sterile glass spreaders. These plates were incubated overnight at 37°C, and up to 10 sorbitol-negative (non-fermenting, colorless) colonies per plate were transferred to MacConkey plates for evaluation of lactose fermentation. Lactose-positive, sorbitol-negative colonies were tested for glucuronidase activity using a 4-methlyumbelliferyl-beta-D-glucuronide (MUG) assay performed in 96 well plates. MUG negative colonies were retested using sorbitol MacConkey and, if sorbitol-negative, were tested by slide agglutination for O157 antigen. *E. coli* O157:H7 isolates were assayed for verotoxin genes using DNA-DNA hybridization.

From every 10th lamb in order of collection, approximately 10 gm of feces were collected and placed into a tube containing 20 ml of TSB. Tellurite (2.5 mg/ml) along with cefixime and vancomycin at rates given above was added to the tube. After arrival at the laboratory, the samples were brought up to 100 ml of TSB (with tellurite, cefixime, and vancomycin) and then incubated at 37°C while being shaken overnight. Serial dilutions to 10^{-5} were prepared and 100 ml of the 10^{-2} and 10^{-5} dilutions were plated onto SMACtct plates also containing 100 mg/ml of MUG. Up to 10 clear, non-fluorescent (i.e., sorbitol-, MUG-) colonies were picked and tested for 0157 with a slide agglutination test using specific O157 antisera. Verotoxin probing was done as described above.

Geographic origin, production system, distance shipped, transit time, lot size, average weight per animal, and time held at the slaughter establishment prior to slaughter were determined for each lot of lambs sampled.

Results

Overall, 8 of 882 lambs sampled tested positive for *E. coli* O157:H7. Of the 882 samples, 160 were swabs and 722 were pellets; 1 of the former was positive and 6 of the latter (0.6% and 0.8%, respectively). One of 90 (1.1%) of the 10 gm samples was found to be positive. This positive sample was not identified as positive through culturing a single fecal pellet. Of the eight lambs found to be positive for *E. coli* O157:H7, six were shedding isolates positive for both VT-I and VT-II. Two lambs were shedding isolates positive for VT-II only.

Table 1 shows the percent of lambs originating from different sources. A majority of the lambs originated from commercial feedlots. Of the 8 lambs positive for *E. coli* O157:H7, seven (88%) were from commercial feedlots.

Table 1. Origin Distribution for Lots of Sampled Lambs.

Origin	Percent
Commercial Feedlot	56
Farm Flock	20
Small Feedlot	24

Table 2 describes the shipping distances traveled by the sample lambs. Among lambs that were transported less than 200 miles, six were positive (prevalence = .68%), while two lambs transported greater than 200 miles were positive (prevalence = .23%).

Table 2 - Frequency Distribution of the Shipping Distance of Lots of Sampled Lambs

Distance Shipped (Miles)	Frequency
1-50	10
51-80	4
81-180	6
181-350	3
>360	3

Transit and holding time data are presented in Tables 3 and 4. Prevalence of *E. coli* O157:H7 was 0.23% in lambs held for less than 12 hours prior to slaughter and 0.68% for lambs held 12 hours or more.

Table 3 - Frequency Distribution of Transit Time for Lots of Sampled Lambs

Transit Time (hours)	Frequency
0.25-1.00	12
1.25-3.00	8
3.25-10.00	3
>10.00	3

Table 4 - Frequency Distribution of Holding Time for Lots of Sampled Lambs

Holding Time (hours)	Frequency
1	10
2	3
3	2
14	1
16	3
17	3
18	4

Combining time in transit with holding time at the slaughter establishment provided the total time from farm to slaughter. The distribution is shown in Table 5. Among 570 lambs with a total time from farm to slaughter of less than 18 hours, the prevalence was 0.23% while it was 0.68% among lambs for which the farm to slaughter time was equal to or greater than 18 hours.

Table 5 - Frequency Distribution of Total Time from the Farm to Slaughter of Sampled Lambs

Total Time (hours)	Frequency
1.25	1
1.50	2
1.75	1
2.00	4
2.50	2
4.00	2
6.00	1
14.00	2
17.00	1
19.00	3
19.50	1
20.00	2
22.00	1
24.00	1
25.00	2

Discussion

Although the limited sample size of the present study prevents conclusions regarding the effect of holding time on the prevalence of *E. coli* O157:H7, the higher prevalence observed among lambs held for long periods is consistent with the effect of holding time. Since holding time is a pre-slaughter practice potentially under management control, the findings of this study should be followed up with a larger study to examine this relationship further.

The additional E. coli O157:H7-positive sheep detected using the 10 gm sample suggests a true prevalence that is somewhat higher than that measured by swab or 1 pellet sample. However, the fact that only 1 of 90

of the 10 gm samples was positive does seem to confirm that the prevalence was relatively low - near 1%.

It is typical in fecal cultures that adding extra procedures will asymptotically increase prevalence since there is no legitimate gold standard which could be expected to have 100% sensitivity. The primary goal in running 10 gm samples on a subset of samples was to protect against concluding a much lower prevalence than actually exists due to possible poor sensitivity of using small fecal amounts.

Previous work in cattle had indicated that swabs are only slightly less sensitive than 10 gm samples and much more amenable to cost-efficient handling in large scale studies. The present data suggest that the same is true for sheep feces.

Fecal Shedding of *Escherichia coli* O157:H7 in Adult Sheep at an Auction Market

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Abstract

The objectives of this study were to determine: (1) if sheep shed *E. coli* O157:H7 in their feces, and (2) if the distance the animal travels in market channels affects the prevalence of shedding. A total of 500 samples were tested. Sheep brought to the auction came from New Jersey, Pennsylvania, New York, Ohio, and Tennessee. Two sheep (0.4%) tested positive for *E. coli* O157:H7.

Purpose

During times of feed deprivation or nutritional stress, rumen pH rises. Such conditions have been shown to allow enteric bacteria to survive and multiply so that rumenal contents may become a reservoir of enteric pathogens. *E. coli* O157:H7 has been shown to grow vigorously in the rumen of cattle held off of feed for 48 hours. Experimental restriction of feed for sheep has been shown to increase fecal excretion of Salmonella and *E. coli*.

Surveys of fecal shedding of *E. coli* O157:H7 have largely been done on cattle on the farm. A need exists to study the effects of marketing practices on the shedding of *E. coli* O157:H7. Field studies on fecal shedding of *E. coli* O157:H7 by sheep have not been done.

The objectives of this study were to determine: (1) if sheep shed *E. coli* O157:H7 in their feces, and (2) if the distance the animal travels in market channels affects the prevalence of shedding.

Materials and Methods

This study was conducted at a livestock auction market in New Jersey. Sheep traveled to the market from local and out-of-state sources. Some out-of-state sheep have traveled through other auction markets prior to arrival at the study site.

Sheep were placed in pens by the consignor, with 6-16 adult sheep per pen. Five to eight sheep older than 1 year were sampled per pen.

A sample of 1-10 grams of feces was collected from each sheep by rectal retrieval. Non-sterile, latex gloves were used for each sheep to avoid cross-contamination during sampling. Samples were emulsified in buffered glycerol saline and transported to the laboratory under refrigeration.

Within 24 hours of collection, 1 ml of sample was transferred to 10 ml of saline and another 1 ml of sample was transferred to 10 ml of Doyle's enrichment media. The saline broth was plated to sorbitol MacConkey plates and incubated for 24 hours at 37°C. Doyle's broth was incubated for 24 hours at 37°C and then plated. These plates were then incubated for an additional 24 hours.

Any sorbitol negative colonies were tested in *E. coli* antisera by slide agglutination. Positive colonies were streaked onto TSA slants and retested in 24 hours using a latex agglutination test. Any positive colonies were then titered out to a 1:320 using the O157 antisera and incubated in a water bath overnight at 48-50°C. All positive isolates were confirmed as *E. coli* O157:H7 using the API identification system.

All positive *E. coli* O157 colonies were tested for H7 antigen using the tube agglutination test. The antigen was prepared by passing the organism through semi-solid media. After enhancement of motility, TSB was inoculated and incubated for 24 hours at 37°C. The broth culture was then preserved by adding 0.5% formalin. The antigen was tested with H7 antisera at a 1:3200 dilution. After 1 hour incubation in a 48-50°C water bath the tube was checked for agglutination.

Sample collection was completed on November 22, 1995. The total number of samples collected was 650. Problems with pH indicator in the sorbitol MacConkey plates used (Difco) required nullification of results from the first 150 samples. Conclusions reached in this study were based on the remaining 500 samples tested.

Results

This study was done on a limited number of sheep. Until September 30, 1995, no samples were culture-positive for *E. coli* O157:H7. At the time of completion of the project on November 22, 1995, two samples were found positive for *E. coli* O157:H7. Both samples were from sheep that originated in Ohio. One sheep appeared normal. The second sheep was thin, had mucus and blood in its feces.

The prevalence of *E. coli* O157:H7 shedding in this study was determined at 0.4%.

Discussion

Sheep sampled in this study were brought to the auction by consignors from the following states: New Jersey, Pennsylvania, New York, Ohio, and Tennessee. Several lots of sheep had backtags from auctions in Ohio.

In this study, *E. coli* O157:H7 was only detected in sheep which had traveled long distances to the auction. Complete tracing on the positive animals was not possible; however, the consignors of both sheep found positive for *E. coli* O157:H7 generally bought sheep at auction in Ohio and sold them in New Jersey. It is therefore likely that the positive animals had been in auction channels for several days prior to testing.

Further Work

Areas for further research might include following sheep or cattle from the farm through market channels to slaughter to determine if there is increased shedding of *E. coli* O157:H7 at any particular point, testing sheep on the farm to determine the prevalence of *E. coli* O157:H7, continuing this study to obtain a larger number of test animals, and performing a study similar to this one on cattle in market channels.

Shedding of *Escherichia coli* O157:H7 by Feedlot Cattle

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Abstract

Intense interest has been focused on *E. coli* O157:H7 since the early 1993 outbreak of *E. coli* O157:H7 infection in several western states. The Cattle on Feed Evaluation (COFE) conducted by the National Animal Health Monitoring System (NAHMS) began collecting data in the fall of 1994 to shed light on the on the distribution of *E. coli* O157:H7. COFE collected fecal samples from 100 feedlots in 13 major cattle feeding states (Arizona, California, Colorado, Idaho, Illinois, Iowa, Kansas, Minnesota, Nebraska, Oklahoma, South Dakota, Texas, and Washington). COFE confirmed findings from other studies indicating *E. coli* O157:H7 occurs in cattle operations at low frequency but is widely distributed. Overall, *E. coli* O157:H7 was recovered from 1.61% of the samples. *E. coli* O157:H7 was most commonly recovered from pens of cattle that had been on feed the shortest time (3.01% of samples), while those that had been on feed the longest were least likely to be positive for *E. coli* O157:H7.

Purpose

E. coli O157:H7 has been the focus of intense interest since the early 1993 outbreak of *E. coli* infection in several western states. The COFE quantified the prevalence of *E. coli* O157:H7 in fecal samples from cattle feedlots. Factors associated with recovery of *E. coli* O157:H7 were evaluated from fecal samples of cattle in the study.

Materials and Methods

In collaboration with the National Agricultural Statistics Service, State and Federal Veterinary Medical Officers, the National Veterinary Services Laboratories, and the Field Disease Investigation Unit of Washington State University, NAHMS collected data on health and management of cattle from a broad-based sample of feedlot operations in 13 major cattle feeding states. In addition, 100 volunteer feedlots were enlisted to provide fecal samples to be tested for *E. coli* O157:H7. These feedlots were distributed across the 13 states in proportion to the number of cattle on feed in those states.

In each feedlot, fecal samples were collected from up to four pens: (a) 1 pen shortest on feed, (b) 1 pen longest on feed, and (c) 2 randomly selected pens, if available. Up to 30 swab samples were collected from each pen from fresh feces on the pen floor. The samples were tested for *E. coli* O157:H7 and the isolates were then probed for genes coding for verotoxin production.

Results

Overall, *E. coli* O157:H7 was recovered from 1.61% of the samples collected (see Table 1). *E. coli* O157:H7 was most commonly recovered from pens of cattle that had been on feed the shortest period of time (3.01% of positive samples). Samples from pens that had been on feed the longest were least likely to be positive for *E. coli* O157:H7 (1.08% of positive samples).

Table 1. Percent of Samples Positive for *E. coli* O157:H7

Pen Type	Number Samples	Number Positive	Percent Positive	Percent of Positives
Shortest	2,988	90	3.01	47.1
Longest	2,963	32	1.08	16.8
Random	5,930	69	1.16	36.1
Total	11,881	191	1.61	100.0

E. coli O157:H7-positive feedlots were widely distributed. Overall, the organism was detected in one or more samples from 63% of the feedlots.

All *E. coli* O157:H7 isolates possessed genetic material to code for the production of one or both of the toxins believed to be important in the pathogenesis of human disease (Shiga-like toxin 1 and Shiga-like toxin 2).

Discussion

Data derived from COFE seem to indicate that the *E. coli* O157:H7 organism is widely distributed in feedlot cattle populations, but the prevalence is low. These findings are similar to other cattle populations that have been evaluated, such as dairy cattle and calves.

The percent of samples positive for *E. coli* O157:H7 in a pen varied from zero to 36.7%. On a feedlot basis, the percent of total samples positive for *E. coli* O157:H7 was from 0 to 10%. The variation in percent of samples positive

per feedlot suggests that the agent may be amenable to reduction through management intervention.

Further Work

The next phase of analysis from COFE data will focus on relationships between management practices (general and nutritional) and number of positive samples. An increased or decreased likelihood of shedding of *E. coli* O157:H7 has been tentatively associated with a variety of management factors in studies of dairy operations.

From these tentative associations, targeted studies could define factors that modulate shedding. Such studies may eventually lead to strategies for decreasing the shedding of the organism.

Salmonella enteritidis

Unpasteurized Liquid Egg Survey for *Salmonella enteritidis*

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Abstract

This survey closely followed the protocol used in the 1991 liquid egg survey. The 20 plants participating were nearly the same as in the 1991 survey. USDA Agricultural Marketing Service (AMS) inspectors collected 8-ounce samples from each participating plant. Sample collection began in October 1994 and was completed in September 1995. Of the 929 samples collected, 177 (19%) were SE-positive. Overall, 48% of the samples were positive for any Salmonella. When compared with the 1991 results, the 1995 results suggest that the frequency of SE isolations from unpasteurized liquid egg has nearly doubled in the Northern and Western regions but is essentially unchanged for the Southeastern and Central regions.

Purpose

In order to evaluate trends in *Salmonella enteritidis* (SE) occurrence in the egg industry, a survey of unpasteurized liquid egg originally conducted in 1991 was repeated in 1995. The Centers for Disease Control (CDC) surveys human SE occurrence through its outbreak and sporadic isolate reporting systems. Other than traceback investigations, the USDA control program has no routine method for evaluating prevalence of SE in laying flocks. Therefore, a national survey of the egg industry every few years is needed. Results of the 1991 survey showed that sampling of unpasteurized liquid egg correlates well with slaughter hen sampling, but at a fraction of the cost of a slaughter plant survey.

The purpose of this study was to determine if the frequency of SE-positive unpasteurized liquid egg samples had changed from that observed in the 1991 survey. In addition, a change in the distribution and occurrence of various SE phage types between 1991 and 1995 was hypothesized.

Methods

This survey was designed to closely follow the protocol established for the 1991 survey. The 20 plants enrolled to participate were nearly all the same as the 1991 survey. As in the 1991 survey, a weekly 8-ounce sample of unpasteurized liquid egg was collected in each plant by USDA Agriculutral Marketing Service (AMS) inspectors. These samples were sent to the National Veterinary Services Laboratories (NVSL) for culturing and results were reported as follows: negative for Salmonella, Salmonella-positive but SE negative, or Salmonella-positive and SE-positive. Phage typing of SE isolates was also completed by NVSL.

The AMS inspectors determined the U.S. region of origin for the eggs sampled. To avoid confusion, inspectors typically sampled storage tanks containing eggs from a single region. Regions were defined using the Veterinary Services boundaries of 1991. Sample collection began in October 1994 and was completed in September 1995.

Results

Of the approximately 929 samples collected during the survey, 177 (19%) were SE-positive. Frequency of SE-positive samples was 39%, 10%, 10%, and 12% in the Northern, Southeastern, Central, and Western regions, respectively. Overall, 48% of the samples were *Salmonella*-positive. Phage type (PT) 13A was the most frequent strain of SE isolated in the samples. It represented 35% of all SE. Phage type 8 (27%) and PT4 (12%) were the next most frequently isolated SE.

Although the frequency of Salmonella-positive (non-SE) samples increased in the summer months, SE isolations showed no seasonal pattern.

Discussion

Results and analysis are still to be finalized. Data validation is not yet complete, therefore all results and conclusions are tentative. Analysis will not likely be completed until early 1996.

When compared with the 1991 survey results, the 1995 results suggest that the frequency of SE isolations from unpasteurized liquid eggs has nearly doubled in the northern and western regions, but is essentially unchanged for the southeastern and central regions. Such results may suggest an increase of SE-positive laying flocks in the northern and western regions. Alternatively, it is possible that flock prevalence is unchanged (or even reduced) in these regions, but the frequency of diverting eggs from SE-positive premises has increased. Other explanations for this increase must also be explored.

In the 1991 survey, PT8 was the predominant strain of SE recovered. The apparent shift of predominant phage types to PT13A in 1995 is noteworthy. It is possible that such a shift occurred due to continuing reintroduction of new strains of SE into commercial laying flocks. Such an interpretation would mean that SE is still spreading in the egg industry and some route of introduction into flocks, in which PT13A is more prevalent, is not being well controlled. Alternatively, it is possible that PT13A is better adapted to survive in poultry environments, and so has competitively replaced PT8. It is also possible that other SE phage types can be transformed into PT13A through some unknown mechanism, so that these results just demonstrate the plasticity of phage types over time.

The lack of an increase in SE-positive samples in the warmer months of the year may be explained by the rigid laboratory protocol used in this study. The number of Salmonella-like colonies selected for serotyping from each plate was limited to no more than three. As the load of Salmonella increased on plates in the warmer months, the likelihood of selecting a SE colony actually decreased. Although the number of colonies per plate was not recorded in this study, such an increase is reasonable to hypothesize given the increased frequency of Salmonella-positive samples noted in the warmer months. However, this explanation will require further analysis of the data.

1995 Spent Hen Survey of *Salmonella enteritidis*

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Abstract

Eight spent hen slaughter plants were enrolled to participate in the survey. Samples were collected in each slaughter plant on a twice a week schedule by VS field personnel, except in one plant where FSIS personnel collected the samples. From each unique flock of spent hens presented for slaughter on sampling days, one cecum from each of 300 hens was collected. Sample collection began in July 1995 and was completed in September 1995. Of 305 flocks sampled, 136 (45%) had one or more SE-positive samples. Flock prevalence was 65%, 17%, 40%, and 23% in the northern, southeastern, central, and western regions, respectively. Results of this survey suggest an increase (or at least no decrease) in the flock prevalence of *Salmonella enteritidis* in the U.S. during the past four years. Noteworthy is the increase in prevalence in the northern region flock of 45% in 1991 to 65% in 1995. All results presented reported here are preliminary.

Purpose

Based on preliminary results of an on-going survey of *Salmonella enteritidis* (SE) in unpasteurized liquid egg, it was decided to repeat a national survey of spent laying hens originally conducted in 1991. From the 1995 unpasteurized liquid egg survey, it appeared that SE occurrence in the egg industry was higher in some regions of the U. S. , and unchanged in others, compared to a similar survey completed in 1991. In 1991, an unpasteurized liquid egg and a spent hen survey were both completed. Their results suggested good correlation between the surveillance techniques. However, the 1995 liquid egg results raised the hypothesis that an increase in frequency of SE-positive samples could be explained by an increase in deliberate diversion of eggs from SE-positive flocks. Although such a management decision could be interpreted as good for public health, it might bias estimates of the occurrence of SE from a liquid egg survey.

The purpose of this study was to determine if the regional frequencies of SE-positive spent hen flocks correlated with those estimated from the liquid egg survey and to obtain an estimate of flock prevalence for western region flocks (this estimate was not vailable from the 1991 survey). The hypothesis is that the distribution and occurrence of various SE phage types isolated from spent hen flocks may have changed from 1991 to 1995.

Methods

This survey was designed to follow the protocol established for the 1991 spent hen survey. Eight spent hen slaughter plants were enrolled to participate in the survey. These were distributed as follows: 3, 2, 1, and 2 in the northern, southeastern, central, and western regions, respectively.

Samples were collected in each slaughter plant on a twice a week schedule by Veterinary Services (VS) field personnel, except in one plant where Fodd Safety Inspection Service (FSIS) personnel collected the samples. From each unique flock of spent hens presented for slaughter on sampling days, one cecum from each of 300 hens was collected. Five ceca were pooled in sterile plastic bags, for a total of 60 pooled sampled per flock. Collectors wore disposable gloves and changed these after each pool was collected.

Laboratory support for each plant was arranged with state or university laboratories with SE culturing experience. Laboratory procedures were the same as those used in the 1991 survey. Up to three Salmonella-suspect colonies on XLT4 agar were tested for typical reactions on TSI slants, then tested with Group D antiserum. Group D Salmonellae were sent to the National Veterinary Services Laboratories (NVSL) for serotyping and phage typing. Total number of pooled samples, the number of Salmonella-positive pools, and the number of SE-positive pools were reported for each flock sampled.

Sample collection began in July 1995 and was completed in September 1995.

Results

Of the seven plants in the 1991 survey, five agreed to participate in the 1995 survey. One slaughter plant in the central region, and another in the Southeastern region, were no longer in business in 1995. Therefore, one large plant in the southeastern region was selected to replace these two plants. While no western region plants were enrolled in the 1991 survey, two plants from this region participated in 1995. Of the seven labs that participated in the 1991 survey, four were available for this survey.

Of 305 flocks sampled, 136 (45%) had one or more SE-positive samples. Flock prevalence was 65%, 17%, 40%, and 23%, in the northern, southeastern, central, and western regions, respectively. The combined central-western region prevalence (as done in 1991) was 33%.

Of 17,961 pooled samples cultured in the study, 918 (5%) were SE-positive. A total of 7206 (40%) samples were Salmonella-positive. Nearly all flocks (98%) had at least one Salmonella-positive sample.

Of 14 different phage types (PT) found in this survey, PT13A was the most common (33%), while PT8 (31%) was slightly less common. Phage type 4 was isolated from 5 flocks in the Western region but represented only 2% of all SE isolates.

Discussion

All results presented here are preliminary. Data validation is not yet complete. Full analysis of the data will probably not be complete until early 1996. Although fewer flocks were sampled in this survey as compared to the 1991 survey, these results suggest an increase (or at least no decrease) in the flock prevalence of SE in the U. S. during the past four years. Noteworthy is the increase in the northern region flock prevalence from 45% in 1991 to 65% in 1995. In light of these results, it does not seem extraordinary that the unpasteurized liquid egg survey results suggest nearly a doubling in the frequency of positive samples in this region between 1991 and 1995. Such a finding would be expected if the prevalence of positive flocks is increased and some more diversion of eggs from SE-positive flocks to pasteurization plants is occurring.

Combined central-western region flock prevalence results are nearly twice those found in 1991. In this 1995 survey, we were able to separate central and western region results and show that prevalence in central region states (40%) is nearly at the level of the northern region in 1991 (45%). In contrast, results for the western region (23%) are more comparable to those observed in the southeastern region (17%) in 1995. The occurrence of PT4 in some western region flocks presents an opportunity to monitor the spread of an emerging phage type across time. Until 1993, PT4 had never been isolated from a poultry flock in the U. S.

Comparison of spent hen and liquid egg results show some discordance, particularly in the central region (Fig. 1). While the 1991 to 1995 trend in the liquid egg survey indicated the central region was essentially unchanged, spent hen survey results show a marked increase in the prevalence of positive flocks

over the same period. Possible explanations include differences in the populations of eggs and spent hens sampled in these surveys, differences in the timing of the surveys (e. g. , seasonal effect), slaughter or pasteurization plant confounders, or laboratory confounders. These explanations need further analysis.

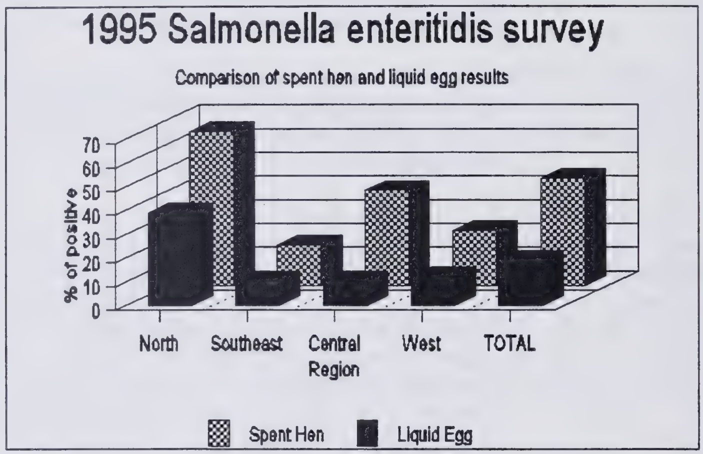

Figure 1. Comparison of regional spent hen and unpasteurized liquid egg survey results.

As in the 1995 liquid egg survey, PT13A was the predominant SE phage type isolated from spent hen samples. This finding requires more analysis but it seems likely that it will support a continual and preferential introduction of PT13A into U. S. flocks.

All conclusions made here are preliminary and subject to final analysis.

Development and Support of the California Egg Quality Assurance Plan

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Purpose

Development of a California Egg Quality Assurance Program was suggested to egg industry leaders following the 1993 series of outbreaks of *Salmonella enteritidis* in several locations in California. Egg industry response to the proposed program was not overwhelming. When APHIS announced in Fiscal Year 1994 its willingness to provide national leadership in the development of preharvest food safety programs, the egg industry was contacted by a contract facilitator who was well known and respected in the California egg industry. The industry leaders responded positively and said they were ready to work on the development of a quality assurance program.

Materials and Methods

Under the leadership of the contract facilitator, a series of meetings were held with the egg industry which led to the development and adoption of the California Egg Quality Assurance Plan in the Spring of 1995. Participants in the meetings included most of the major egg producers in the state, the California Department of Food and Agriculture, the Animal and Plant Health Inspection Service (APHIS) and the Agricultural Marketing Service (AMS) of the U.S. Department of Agriculture, the University of California at Davis Cooperative Extension Service, the Veterinary Diagnostic Laboratory System, the California Department of Health Services, and the U.S. Food and Drug Administration.

Results

The development and adoption of the California Egg Quality Assurance Plan (CEQAP) occurred in the last two years. CEQAP is a producer-oriented animal production food safety program designed to ensure the highest quality and safety of eggs. The program contains 20 core components which form the basis of a Hazard Analysis Critical Control Points (HACCP) plan. Training, record keeping, and research are integral components in documenting the success of the plan.

Under the plan, participants design a monitoring plan applicable to their specific operations. The California Department of Food and Agriculture veterinarians will review farm and processing facilities to ensure compliance with the program components.

Core Program Components

Administrative

- Develop a farm/premises flock egg quality assurance plan.
- Designate an employee or employees as the official quality control supervisor(s) for in-house operations and for follow-up training.

Production

- Purchase chicks and pullets from hatcheries participating in the National Poultry Improvement Plan (NPIP), U.S. Salmonella Enteritidis Monitored Program or equivalent state plan. Chicks should be delivered with a certifying letter. Starter pullets must be obtained from sources with an acceptable Salmonella prevention and control program.
- Transport chicks and pullets in coops and trucks that are decontaminated between flocks.
- Obtain feed from mills that follow accepted feed industry guidelines contained in the *Good Manufacturing Practices and the Recommended Salmonella Control for Processors of Livestock and Poultry Feeds*, 1988, of the American Feed Industry Association (AFIA) or an equivalent program.
- Use animal protein ingredients originating from rendering plants participating in the Animal Protein Producers Industry (APPI) Salmonella Reduction Education Program or equivalent.
- Administer medications, feed additives and pesticides according to approved label directions.
- Maintain an effective flock health program to include vaccinations, monitoring and periodic necropsy of mortality or cull birds.
- Maintain a farm rodent monitoring and reduction program.
- Clean and disinfect pullet and layer buildings before restocking. Third-party visual inspection of cleaning and disinfection is required. The inspection must be done by a certified quality control employee designated by the owner, or by a certified independent professional.

- Utilize a biosecurity plan and train employees on proper procedures to execute the program. Document employee training and comprehension annually.

Processing

- Facilities and equipment must be kept clean and in good repair and shall be completely washed at the end of each day's operation.
  - Provide adequate lighting to properly identify egg defects in the candling booth and the processing area.
  - Use potable water with less than 2 ppm of iron.
  - Maintain wash water at 90°F or higher and at least 20°F higher than the temperature of the eggs to be washed.
  - Use USDA-approved cleaning compound in the wash water.
    - Add and replace wash water continuously every four hours.
  - Spray-rinse washed eggs with warm water and a USDA-approved sanitizer.
- If eggs are oiled, follow USDA guidelines:
  - Refrigerate eggs according to applicable federal, state or local laws.
  - Label egg cartons and cases with a "Keep Refrigerated" descriptor to educate consumers about perishability.
  - Label egg cartons and loose pack eggs with a Julian pack date to assist with product rotation. An optional "sell by" date may be used at the discretion of the packer as long as the sell date does not exceed the date of packing by 30 days.
  - Label cartons and cases with plant of origin number and, if possible, with a flock identification number.
  - Wash and sanitize plastic egg flats after each use or return to the originating farm to avoid cross contamination. Since fiber egg flats cannot be sanitized, they must be returned to the farm of origin.
  - Do not reuse egg cartons and soiled fiber flats.
  - Retail returns shall not be reprocessed for retail shell egg sales.
  - Label eggs with a quality assurance seal only if produced in California by producers participating in the California Egg Quality Assurance Plan.

CFDA will be responsible for validating the producer flock plans. The UC-Davis Extension Service developed a training program to support the California Egg Quality Assurance Program. The training plan consists of four parts: (1) preparing a quality assurance plan, (2) flock health management, (3) cleaning, disinfection, and biosecurity, and (4) egg handling. This training will be required for producers who wish to join the plan.

Pennsylvania *Salmonella enteritidis* Pilot Project

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Abstract

The *Salmonella enteritidis* Pilot Project began in 1992. The egg positivity rate was found to be around 2.75 per 10,000. Epidemiologic research from this project suggested risk factors such as mouse infestation, molting, and increased age of flock. Analysis of the sampling protocols gave helpful information. For example, blood spot eggs are more likely to be SE-positive, flocks with greater than 50% positive manure sample were more likely to have positive eggs, and testing of manure pits provides a better measure of SE status than egg belt testing. Information from the pilot project was used to develop the industry-led Pennsylvania Egg Quality Assurance Program.

Purpose

The *Salmonella enteritidis* Pilot Project started in April 1992 as a cooperative effort by the poultry industry in Pennsylvania, the Pennsylvania Department of Agriculture, the University of Pennsylvania, Pennsylvania State University, and the U.S. Department of Agriculture. The goal of the project was to reduce the SE threat to public health by reducing the number of SE contaminated eggs through a carefully controlled field research program. It involved volunteer egg producers and federal, state, and university personnel. After the program was moved to FSIS, APHIS continued to provide analytical assistance through the Centers of Epidemiology and Animal Health (CEAH). The objective was to develop effective and efficient monitoring procedures that will prevent SE from contaminating eggs.

Materials and Methods

Participating egg producers agreed to having their flocks tested for SE and to carry out a series of control procedures designed to prevent SE or eliminate it from their flocks. These included rodent control, cleaning and disinfection between flocks, and implementing effective biosecurity measures. Eggs from

SE-positive flocks were to be diverted to pasteurization. By February 1994, 76 houses and 134 flocks had been monitored.

Results

It is thought to be practically impossible to eliminate SE from a flock unless the mouse population is significantly reduced. Cleaning and disinfection appears to be important in reducing the pathogen load in a poultry house but does not necessarily eliminate SE from the environment.

Heavy mouse infestations were found to significantly increase the likelihood that a house was environmentally positive.

It was hoped at the outset of the project that it might be possible to relate certain management practices with the presence or absence of SE. Although no striking correlation was noted, generally, the presence of SE seemed to be related to the quality of biosecurity and the presence of mice in the hen house. With few exceptions, the presence of SE in an egg-layer flock did not appreciably increase the rate of morbidity or mortality or affect egg production.

It was expected that a higher level of SE positivity in eggs might be found in young laying flocks when they were first exposed to a contaminated hen house environment, or to the stress related to onset of egg production. However, the reverse was seen: the percent of positivity was highest in the eggs from older birds. This may have some relation to the practice of molting, which was found to be a risk factor of SE. Molting of previously SE-positive flocks appears to be associated with a higher percentage of positive eggs. Although it was expected that SE positivity in the manure and the eggs would be higher in the summer months, no seasonal trends were seen, and the increase in human outbreaks in the warm season appears to be related to other factors. The testing of manure pits and manure scrapers provides a better measure of the SE status of the current flock than sampling of the egg belts, although the difference is not large.

Although sampling of the environment is widely used as a screening method to detect the presence of SE in layer houses, repeated environmental testing of positive houses showed significant variability.

Blood spot eggs were found to be twice as likely to be SE-positive than regular nest run eggs. The use of blood-spot eggs may possibly provide a better way to detect positive eggs than using nest run eggs, but these eggs are not always available for collection in all flocks. Dirty eggs that are washed by immersion were slightly more likely to be SE-positive. A number of other Salmonella

serotypes were found as a result of egg culture, although it is not clear if this was due to shell contamination or actual prior contamination of the egg contents.

The egg positivity rate was found to be 0.0275% or 2.75 eggs positive in 10,000 eggs, from eggs from environmentally positive houses. About 50% of the houses in the project were environmentally positive and eggs from about 50% of these houses were positive for SE at some time during the life of the flock. Flocks with 50% or more manure samples positive for SE were more likely to have positive eggs.

Discussion

The Pennsylvania Egg Quality Assurance Program began in February 1994 using many of the same control procedures and testing protocols developed in the pilot project. Pilot project studies continue with flocks in the Pennsylvania Egg Quality Assurance Program. Additionally, analyses continue with the vast amount of data generated by the pilot project.

Other Food Safety Topics

Salmonella Shedding by Feedlot Cattle

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Abstract

Salmonellae species have been associated with illness among animals, including man. Data from the Cattle on Feed Evaluation indicate that *Salmonella spp.* isolates from cattle do not correspond well with serotypes most commonly associated with human illness. Results show that cattle th: have been on feed longer appear more likely to shed *Salmonella spp.* The sample prevalence of Salmonella within feedlots appears to be highly variable and was near zero or zero in about two-thirds of the feedlots, such that it was not detectable based on a sample size of 50 fecal samples. Shedding of *Salmonella spp.* serotype commonly associated with human illness by cattle in this study occurred infrequently.

Purpose

The National Health Monitoring System (NAHMS) conducted a study of health and management of cattle in feedlots as part of the Cattle on Feed Evaluation (COFE). One of the objectives of the study was to determine the prevalence of Salmonellae in fecal samples collected in feedlot pens.

Materials and Methods

A stratified random sample of feedlots from the 13 major cattle feeding states was selected for COFE. The 13 states are: Arizona, California, Colorado, Idaho, Illinois, Iowa, Kansas, Minnesota, Nebraska, Oklahoma, South Dakota, Texas, and Washington. These states account for more than 85% of the cattle on feed in the United States. Four hundred ninety-eight feedlots with at least 1,000 head carrying capacity responded to the survey. Out of the 498 feedlots that responded, 100 volunteer feedlots were enlisted for the collection of feces to be evaluated for Salmonella spp. In each feedlot, 25 samples were collected from fresh feces on the floor in each of the two cattle pens. Fecal culturing was done at the National Animal Disease Center (NADC). Salmonella isolates were serotyped at the National Veterinary Services Laboratories (NVSL).

The two pens identified for sampling were those cattle that had been on feed the shortest and the longest periods.

Results

Overall, *Salmonella spp.* were recovered from 5.5% of the samples collected (see Table 1). Table 1 shows that compared to pens on feed the shortest amount of time, twice as many samples from cattle that had been on feed the longest were positive for *Salmonella spp*. This finding may indicate that more animals become infected with, or shed, *Salmonella spp.* as the animals are housed together over time. Further analysis may determine if management factors in the late feeding period are more conducive to shedding of the *Salmonella spp.* organism. The positive samples came from 38 of the 100 feedlots with no apparent clustering of positive feedlots by region. A single serotype of *Salmonella spp.* was identified in 16 feedlots. Multiple serotypes were isolated in 22 feedlots. Overall, 26 serotypes were identified.

Table 1. Frequency of Recovery of Salmonella Spp. from Samples Collected in 100 Feedlots by Pen Type

Time on Feed	Feedlots		Samples		
	Number Positive	Percent Positive	Number Collected	Number Positive	Percent Positive
Shortest	25	25.0	2,482	88	3.5
Longest	27	27.3	2,495	185	7.4
All	38	38.0	4,977	273	5.5

Table 2 shows the five most common serotypes of Salmonella recovered from the samples in this study. For comparison, *S. typhimurium, S. dublin, S. typhimurium var. copenhagen, S. cerro*, and *S. newport* were the most common isolates associated with cattle illness for October 1990 to September 1991. The isolates were from on-going Salmonella serotyping services provided by NVSL and animals with clinical disease. The lack of agreement between these results should not be surprising since the isolates from COFE were not preferentially collected from animals that were ill. The 1991 Salmonella Surveillance System Annual Summary published by the Centers for Disease Control and Prevention (CDC) indicate that the five most common *Salmonella spp.* isolates implicated in human illness were: *S. typhimurium, S. enteritidis, S. heidelberg, S. hadar,* and *S. newport.* Isolates were from all human Salmonella cases reported regardless of suspected source of infection. There was no agreement between the serotype and the five most common isolates from the COFE study.

Table 2. Five Most Common Serotypes of *Salmonella spp.*
Recovered from 100 Feedlots

Salmonella Serotype	Number of Isolates	Percent of All Positive Isolates
Anatum	78	27.9
Montevideo	36	12.9
Muenster	33	11.8
Kentucky	23	8.2
Newington	12	4.3
Total	182	65.1

Further Work

Future analyses of data derived from COFE will focus on management factors associated with increased shedding of Salmonella organisms.

NAHMS Swine '95 Grower/Finisher Study

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Purpose

The National Animal Health Monitoring System (NAHMS) conducts periodic national observational studies and uses surveillance programs that provide information on trends in production management practices and animal health. One of the two studies implemented in Fiscal Year 1995 is the Swine '95 Grower/Finisher study.

Specific objectives of the study were related to food safety, including efforts to describe the prevalence of foodborne pathogens on U.S. swine farms (Salmonella, *E. coli* O157:H7, *Yersinia enterocolitica*, Campylobacter) and to identify factors in the shedding of Salmonella.

Material and Methods

The target population for the Swine '95 Grower/Finisher study were producers in the top 16 pork producing states who had at least 300 market hogs. This target population represented 91% of the U.S. hog inventory and 84% of U.S. pork producers. Using a multistage stratified sampling design, the National Agricultural Statistics Service (NASS) identified 540 producers for the study. Veterinary Medical Officers (VMO's) signed up 418 producers to begin on-farm interviews in July 1995.

Management and health questionnaires were completed and biological samples were collected during two on-farm visits. The first visit was completed between July 17 and September 15, 1995. The second visit was completed after one complete turn in the grower/finisher (November 6, 1995 and January 19, 1996). Samples collected on the farm by VMO's include feed, feces, and blood.

Fecal samples will be used to identify the presence of Salmonella in herds and estimate national animal level prevalence. The study will also determine the percent of pre-market hogs in a herd shedding Salmonella and estimate national herd prevalence. To determine if shedding of *E. coli* O157:H7 occurs in the U.S. hog population, 3000 samples were needed to detect the presence of the organism at a level of at least 0.1% prevalence with 95% confidence.

Up to 50 fecal samples were collected from a total of 160 farms, resulting in the collection of 8,000 fecal samples to be evaluated for the presence of Salmonella organisms. Fecal samples were collected from up to 10 pens per farm. Pens containing late finisher hogs (hogs slated for market within 30 days) were selected for the study. Samples of feed fed to late finishing hogs (five 100 gm-samples per farm) will be tested for Salmonella. The samples have been sent to the National Animal Disease Center (NADC) and the National Veterinary Services Laboratories (NVSL) for identification of the group, type, and antimicrobial resistance patterns for Salmonella isolates.

Results

Results are not available yet for Salmonella. The following table contains results for the other food-borne pathogens in fecal samples collected during the first visit.

Pathogen	Number of Farms	Number of Samples	Positive Samples
Arcobacter	13	540	189
Campylobacter spp.	74	3272	785
Y. enterocolitica	56	2436	325
E. coli O157:H7	77	2226	0

Second visits for the NAHMS Swine '95 Grower/Finisher study are currently underway. Majority of the testing results are expected to be available to CEAH staff in March 1996. Fecal samples collected during the study will reveal important information regarding factors affecting the shedding of Salmonella and permit description of antimicrobial susceptibility patterns.

National Trichinae Research Project

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Abstract

The major objectives of the project are to: (1) validate the ELISA as a potential trichinae screening tool, (2) find out if risk factors previously considered important for herd infection remain so, (3) determine if risk mitigation strategies can be implemented to clear a herd of trichinae and remain clean over an extended period, and (4) determine the national prevalence of trichinosis in the U.S. swine herd. Projects are being carried out in three VS areas (New England, New Jersey, and Ohio). In both New England and New Jersey, blood samples are being collected on-farm from 30 licensed waste feeding operations, 30 non-licensed waste feeding operations, and 30 grain feeding operations. A brief questionnaire is completed for each premises. Samples are forwarded to the Agricultural Research Service (ARS) laboratory in Beltsville, Maryland for testing using an Enzyme-Linked Immunosorbent Assay (ELISA). When possible, tissues from serologically positive animals will be subjected to digestion to help determine the accuracy of the ELISA. The Ohio project takes advantage of the slaughter swine surveillance system. Sera from identified, Ohio-origin cull sows and boars slaughtered in Ohio are tested at the Ohio Department of Agriculture Diagnostic Laboratory using a commercial ELISA test kit. Four (5.4% of herds) relatively small herds had at least one positive animal. Approximately 44 herds and 1300 hundred slaughter samples have been tested in New Jersey. Preliminary results indicate the following: (1) low levels of trichinae still exist in domestic swine herds, (2) waste feeders are not more likely to be infected, (3) ELISA is a very sensitive test of infection, and (4) animals that are infected have very low larvae per gram.

Purpose

Trichinosis in swine has affected the pork industry in three significant ways: (1) as a very small, but important, public health concern, (2) as a public perception problem that causes consumers to overcook or even avoid pork, thus preventing pork from realizing its full domestic market potential, and (3) as a trade barrier for exports. Several times in the past, the pork industry has attempted to resolve these problems, but no comprehensive strategy was developed. Today, due to heightened interest in food safety, opportunities

for increased exports of chilled pork created by GATT/NAFTA, and intense competition from beef and poultry for the consumer dollar, the pork industry is renewing its efforts to reduce the impact of trichinosis on the safety, sales, and consumption of U.S. pork.

In response to the above concerns, several organizations (APHIS:VS, the National Pork Producers Council, Agricultural Research Service, and Food Safety Inspection Service), state veterinarians, and others formed the National Trichinae Research Project (NTRP). The major objectives of the project are to: (1) validate the ELISA as a potential trichinae screening tool for use in any future control or certification program, (2) find out if risk factors previously considered important for herd infection remain so, (3) determine if risk mitigation strategies can be implemented to clear a herd of trichinae and remain clean over an extended period, and (4) determine the national prevalence of trichinosis in the U.S. swine herd. In all cases where a positive herd is identified, owners will be offered the opportunity to rid their herd of trichinae infection.

Materials and Methods

Projects are being carried out in three VS areas (New England, New Jersey and Ohio). In both New England and New Jersey, blood samples are being collected on-farm from 30 licensed waste feeding operations, 30 non-licensed waste feeding operations, and 30 grain feeding operations. Sample sizes are such that there will be at least a 95% probability of detecting at least one positive animal if the prevalence is 5%, which means that a maximum of 59 animals will be sampled on any one premises. Sampling is not random, but emphasizes older animals to increase the probability of detecting positives, if present. A brief questionnaire is completed for each premises. In addition, a limited number of slaughter samples are being collected in New Jersey.

Samples are forwarded to the Agricultural Research Service (ARS) laboratory in Beltsville, Maryland for testing using the Enzyme-Linked Immunosorbent Assay (ELISA). If positive sera are found, another visit is made to the premises in order to obtain more samples and if possible to purchase the positive animal. When possible, tissues from serologically-positive animals will be subjected to digestion to help determine the accuracy of the ELISA.

The Ohio project takes advantage of the slaughter swine surveillance system. Sera from identified, Ohio-origin cull sows and boars slaughtered in Ohio are tested at the Ohio Department of Agriculture Diagnostic Laboratory using a commercial ELISA test kit. The samples are then forwarded to the ARS

laboratory to be treated the same as New England and New Jersey samples. Positive sera are traced to the farm of origin and a request is made to do on-farm sampling. The questionnaire is also administered at this time. Again, the results of the on-farm testing and the comparison of results from the commercial ELISA and the ARS ELISA will help in establishing the accuracy of the ELISA.

In all cases where a positive herd is identified, owners will be offered the opportunity to rid their herd of trichinae infection. Each protocol will be unique to the herd, but in general include an assessment of the source of infection (rodents, wildlife, cannibalism, feeding uncooked pork, etc.) Depending on the source of infection, a plan will be developed to clean up the herd. Periodic testing will be conducted to determine the effectiveness of the cleanup plan.

Results

As of September 30, 1995, most of the on-farm sampling in New Jersey and New England had been completed. Work with positive herds is beginning.

Approximately 84 herds (3768 samples) have been tested in New England. Approximately 46 herds and 2058 slaughter samples have been tested in New Jersey.

As of September 30, 1995, approximately 3,000 slaughter samples have been tested at the Ohio diagnostic laboratory and at ARS. Very few positives have been found. Since September 30, 1995, several more positive samples have been found in Ohio and traceback is in progress.

Discussion

Preparatory activities (organizing and setting up) consumed much of the work on the projects in FY 1995. Preliminary results indicate the following:

- Swine herds in the U.S. are still infected, albeit at very low levels, with trichinae.
- Waste feeders are not more likely to be infected.
- ELISA is a very sensitive test of infection.
- Animals that are infected have very low larvae per gram.

Future Work

Work remaining includes completion of on-farm testing in New England and New Jersey, completion of the slaughter sample testing in Ohio, continued testing in the infected herds, cleanup attempts, case-control study, ELISA validation and expansion of the economic study, and execution of the national prevalence study. Finding funding for the remaining work has been problematic.

If enough positive herds are found, a case-control study will be initiated to determine risk factors for infection. Case herds will be matched with a neighborhood control. A detailed questionnaire will be administered to both groups. Odds ratios with 95% confidence intervals will be calculated for putative risk factors.

Finally, a national prevalence study is being planned using sera specifically collected for the project. Two populations of swine will be sampled separately, cull sows and boars and market hogs. 40,000 animals will be sampled in each population. This will provide a .01% confidence interval if the true prevalence is expected to be .01%. The sampling frame will include all federally-inspected slaughter plants in the U.S. Sample size will be proportional to the plant size.

The national prevalence survey is in the planning stage, with initiation scheduled after the completion of the NAHMS Swine '95 serologic studies. In addition to the above, an economic study of the effects of trichinae on the domestic and international markets is complete and may be expanded later.

Future endeavors should include development of a trichinae certification program for swine producers, securing official test status for the ELISA or other tests, international efforts to make a scientific case for acceptance of U.S. pork imports, and seeking out niche reservoirs in the U.S. swine herd.

Needs Assessment for Food Safety Issues in the Broiler Industry

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Abstract

This needs assessment was conducted in preparation for national monitoring efforts in the broiler industry. Phase I explored existing data sources. The National Poultry Improvement Plan (NPIP) data were an excellent resource for looking at specific disease trends in breeder flocks. NPIP monitors Pullorum disease (*Salmonella pullorum*), Typhoid (*Salmonella gallinarium*), *Salmonella enteritidis*, MG (*Mycoplasma gallisepticum*), and MS (*Mycoplasma synoviae*) for breeder flocks of all feather types, and MM (*Mycoplasma meleagridis*) for turkey breeders only. Phase II focused on developing, mailing, and summarizing a broad-based questionnaire to assess issues important to the broiler industry. Public misconceptions regarding food safety and Salmonella were identified as major food safety issues. Phase III activities involved summarizing the 1983-1994 Food Safety Inspection Service condemnation data.

Purpose

The purpose of this project was to establish information needs of the broiler industry and determine how the National Animal Health Monitoring System (NAHMS) might best meet those needs.

Phase I Assessment Activities

Existing Data Sources

Phase I needs assessment activities began in 1994. In the spirit of "reinventing government" and to avoid duplication, an effort was made to determine what sources of poultry health data are available for use as a national monitoring tool. Veterinary Services (VS) personnel in major poultry producing states were asked to summarize existing sources of poultry health data in their respective states. Responses were mailed to the Centers for Epidemiology and Animal Health (CEAH) or presented at the NAHMS Monitoring and Surveillance Workshop held in Fort Collins in March 1994. Existing data sources were identified and potential areas for study were suggested.

The objectives of poultry monitoring as outlined by VS personnel are as follows:

- Focus on answering specific and realistic questions.
- Limit to NON-REGULATORY diseases.
- GUARANTEE CONFIDENTIALITY.
- Provide valuable response.
- Address issues with largest impact on the industry (e.g., food safety).
- Get information to people who can use it (e.g., veterinarians, service personnel, growers).

Potential benefits to the industry include:

- Information on production and disease within area and in other regions of the U.S.
- Information on high impact areas for the industry (e.g., food safety).
- Information on risks to current production levels.
- Provide epidemiologic services to the industry.

Existing data sources and their limitations were discussed. There is an apparent need to combine data from several sources in order to capture an accurate picture of the health status of the national poultry flock.

National Poultry Improvement Plan (NPIP) Data

The NPIP data are an excellent resource for looking at specific disease trends in breeder flocks. The program started in 1934 for chickens and in 1943 for turkeys. The objective of the program is to identify breeder flocks infected with select vertically transmitted diseases and eliminate these flocks from the breeding population. The scope of the program has changed over time, but essentially all breeder flocks that ship eggs across state lines participate in the program. The program monitors pullorum disease (*Salmonella pullorum)*, typhoid (*Salmonella gallinarium)*, *Salmonella enteritidis*, MG (*Mycoplasma gallisepticum)*, MS (*Mycoplasma synoviae)*, and MM (*Mycoplasma meleagridis)* (turkeys only) for breeder flocks.

The NPIP data were summarized from 1971 to-date looking specifically at the number of birds monitored, the percentage of the national flock monitored, and the percentage of the national flock positive for these program diseases. Essentially 100% of the national broiler breeder flock is monitored for pullorum and typhoid. Few were found positive in the national broiler

breeder flock for both diseases. The percentage of broiler breeders monitored for MG has increased from 20% in 1971 to nearly 70% in 1994. The percentage of broiler breeders monitored for MS has increased from less than 10% in 1975 to nearly 70% in 1994.

Phase II Assessment Activities

Questionnaire to Assess Important Issues

Phase II needs assessment activities involved developing, mailing, and summarizing a broad-based questionnaire to assess issues important to the broiler industry. A one-page front and back questionnaire was developed and mailed with an endorsement by the National Broiler Council to all members in 1995. Thirty responses were returned and summarized. Food safety was identified as the number one issue faced by the broiler industry. Environmental issues and broiler health ranked second and third respectively.

The major food safety issues identified by the survey were public misconceptions regarding food safety and Salmonella. Environmental issues deemed important to the broiler industry were waste disposal and point source contamination. Broiler health issues ranked in order of importance were immunosuppressive disorders, inflammatory process, gangrenous dermatitis, infectious laryngotracheitis virus, and infectious bronchitis virus.

Other issues important to the industry included: market expansion issues such as development of new products and export barriers; quality assurance issues such as shelf life, Hazard Analysis Critical Control Points (HACCP) and product wholesomeness; and human resource issues such as labor availability, OSHA regulations, and repetitive motion disorders in people. While animal welfare issues were a concern, the majority of respondents felt that misconceptions surrounding the growing of broilers was the number one issue.

When asked what disease should be considered "Disease of the Year" for 1994 on a company basis, the diseases most often mentioned were: laryngotracheitis virus, airsaculitis, gangrenous dermatitis, inflammatory process, and infectious bronchitis virus. For the industry as a whole, gangrenous dermatitis and laryngotracheitis virus were the top two contenders for "Disease of the Year."

When asked for the most appropriate measures for tracking the health status of the national broiler flock, the overwhelming responses were FSIS data and diagnostic laboratory data.

Input from Veterinary Services (VS)

In addition to the VS input in identifying existing data sources, VS personnel were contacted following the industry questionnaire in order to keep them informed of important issues and get their feedback. The questionnaire results were summarized for VS personnel and letters containing the summaries were mailed to the regional directors along with a request for any input from the major broiler producing states. Most states deferred to the industry questionnaire although some provided added suggestions.

Input from Southeastern Poultry and Egg Association

Southeastern Poultry and Egg Association conducts an industry survey every year to determine and prioritize research needs. A number of categories are included in this survey related to genetics, feed mill operations, management practices for breeders, hatcheries and broilers, live haul, processing, etc. A number of research priorities related to food safety are listed under these categories in addition to a specific category for food safety research needs. For 1995, research needs identified by the industry related specifically to preharvest food safety issues included:

- Determine effectiveness of "Nurmi" undefined flora for competitive exclusion in broilers administered in hatchery.
- Devise prevention strategies for Campylobacter infection of broilers.
- Define role of contaminated feed in colonizing broilers with Salmonella.
- Define role of Salmonella colonized breeders in producing colonized broilers at processing.

Phase III Assessment Activities

FSIS Condemnation Data Summary

Phase III needs assessment activities involved summarizing the FSIS condemnation data from 1983 through 1994. The purpose of this study was to identify specific diseases that can be monitored using the FSIS condemnation data. All condemnation categories were reviewed for trends. Only those plants which operated consistently from 1983-1994 were included in the summary. The data were divided into regions with an effort to have equal numbers of processing plants in each region.

The data revealed that the number of broilers inspected have steadily increased over time as has the weight and size of the broilers inspected. Condemnation rates have fluctuated greatly by region over time with certain regions exhibiting consistently high condemnation rates.

Data for the top five broiler producing states and Delmarva (Delaware, Maryland, and Virginia) were also summarized. Trends were the same as for the national data with the exception of condemnation rates. Though the rates fluctuated similar to those at the national level, there were no specific states that were consistently above or below average overall. There were some state-specific differences within certain condemnation categories, but these were attributed to the types of birds grown rather than a cluster of specific disease problems.

The two condemnation categories that accurately reflected a disease process was the leukosis condemnation which is an indicator of Marek's disease in young chickens and the squamous cell carcinoma condemnation. All other condemnation categories were "catch-all" categories or could contain birds affected with a variety of specific diseases.

One interesting finding is that when the incomplete 1995 data were graphed, condemnations due to leukosis were markedly up in 1995. This led to dialogue regarding an atypical Marek's virus and ultimately resulted in Marek's disease becoming part of the focus of the 1996 broiler study.

Overall Impressions and Plan

The broiler industry does not need the type of information routinely collected in NAHMS studies. This needs assessment identifies many potential areas needing research. With the exception of the prevalence studies suggested, this assessment identified a need for more focused epidemiologic studies. In an attempt to meet their needs, part of the 1996 broiler study will address the Marek's disease problem using a case-control study to identify risk factors associated with infection and increased condemnations.

A detailed diagnostic laboratory survey will also be conducted as part of the 1996 broiler study. The focus of the survey is to determine how representative diagnostic laboratory data are of the health status of our national flock. In addition, we hope to document current levels of surveillance for Office of International Epizootics (OIE) list A, list B, and list C diseases.

Development and Support of the California Dairy Beef Quality Assurance Program

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Abstract

This project began in response to an urgent concern about excessive antibiotic residues in California cull dairy cows and bob veal calves. A task force of industry and agency leaders was formed under the leadership of Veterinary Services and California Department of Agriculture Animal Health Branch. This task force designed and implemented the California Dairy Beef Quality Assurance Program in Southern California, also known as the 10-Point Plus Plan. Under the plan, state and federal animal health personnel were asked to support three main functions: (1) a premises-based individual animal identification program, (2) immediate traceback and educational visits to the dairy determined to be the source of new antibiotic residues, and (3) field studies to solve the residue problem. Currently, state and federal animal health personnel maintain the Dairy Beef Quality Assurance Backtag System in Southern California and utilize the regular brucellosis market backtag identification in the rest of the state. There are about 300 new residue violation cases from about 600,000 cows slaughtered (beef and dairy) being followed up each year. This is an apparent residue rate of 1 in 2000 (0.05%) slaughter cows. Completion of the field study for testing the antibiotic residue test kits and the regulatory test system in California is scheduled for January 1996.

Purpose

This project began in 1990 in response to an urgent concern about excessive antibiotic residues being found in cull dairy cows and bob veal calves at slaughter in California. The USDA Food Safety Inspection Service (FSIS) Regional Residue Staff, the Food and Drug Administration (FDA) Pacific Region Compliance Office, and the California Department of Food and Agriculture (CFDA) Feed Fertilizer & Livestock Drugs Branch expressed this concern to the California dairy industry. Consequently, a task force was formed under the leadership of Veterinary Services and the CFDA Animal Health Branch managers. These CFDA managers were responsible for successfully eradicating brucellosis from the Chino dairy population.

Materials and Methods

Two teams were formed: an "Industry Team" and an "Agency Team." The Industry Team was made up of volunteers including dairy producers, dairy veterinary practitioners, University of California (UC) Extension Service, UC-Davis Veterinary School, state and federal animal health veterinarians, and other allied industry. This team designed and implemented the California Dairy Beef Quality Assurance Program, also known as the 10-Point Plus Plan, in Southern California.

State and federal animal health personnel were asked to support three main functions: (1) a premises-based individual animal identification program using a specially designed backtag applied at the dairy by the producer, (2) immediate traceback and follow-up visits to the dairy determined to be the source of new antibiotic residues, and (3) field studies to solve the residue problems. The first targeted problem was to perform the residue tests on both the farm test kits and the regulatory monitoring tests.

Implementation of the Dairy Beef Quality Assurance Program in Southern California began on October 1, 1992, which received complete cooperation and support. After a year of successfully reducing violative residues, the plan was recommended to the dairy industry for implementation in the remainder of the state. Although the 10-Point Quality Assurance Plan and premises-based backtag was not adopted in Northern California, the traceback and educational visits aspect of the plan were accepted and implemented by the beginning of fiscal year 1994. Major dairy organizations in California have formed a statewide Dairy Quality Assurance Committee. This committee will redesign the Dairy Quality Assurance Program beginning in February 1996 based on the outcome of field studies.

Results

State and Federal Animal Health personnel maintain the added Dairy Beef Quality Assurance Backtag System in Southern California and utilize the regular brucellosis market backtag identification in the rest of the state. The dairy industry has not decided whether or not to use the premises-based backtag statewide.

All antibiotic residue traces recommended by FSIS for educational visits on the dairies is being continued statewide. There are about 300 new residue violation cases each year being followed up from about 600,000 cows (beef and dairy) slaughtered. This is a residue rate of 1 in 2000 (0.05%) slaughter cows, if we assume all the residues are being detected by the current USDA inspection and sampling procedure. Studies of the effectiveness of the USDA

inspection in California support the previous assumption that very few residue violations are being missed in the state.

Discussion

Much has been accomplished in the area of cooperative voluntary approaches to solving food animal industry problems in California in the past four years. It has been a "win-win" relationship with both industry and government.

While there has been no ground swell of enthusiasm from dairymen to create a new program, most dairies are willing to try reasonable approaches to correct record keeping and drug management practices.

The regulatory agencies involved in the antibiotic residue issues have accepted Quality Assurance Program as positive and helpful. The relationships which were established with dairy producers and their practicing veterinarians have been very productive. Everyone involved genuinely appreciates the timely and direct communication of the antibiotic residue results.

Cooperation and communication with FSIS is open and continuous. The agency team meets regularly to update each other, consider projects, and focus on significant issues. FDA and CDFA compliance personnel particpate regularly in the meetings and continue to support cooperative efforts. Particular attention is given to preventing interference with compliance actions or duplication of other functions.

Although progress has been made in reducing residues, much remains to be done. Cooperative voluntary approaches are the most effective in the long term; however, participants must be willing to accept the time and effort it requires to bring everyone on board as full partners.

It is possible to gain the cooperation and participation of dairy owners and managers, but it is not as clear how to get adequate information and training to dairy employees. These employees perform many of the treatments that result in violative residues in dairy beef. Creative and innovative educational efforts are needed. Substantial economic incentives either as a reward or a penalty have been suggested to achieve elimination of antibiotic residues in dairy beef.

The studies completed thus far strongly indicate that the field test kits for antibiotic residues in urine are of little or no value in managing residues on dairies. However, the laboratory based regulatory tests appear to be accurate

in identifying dairy beef with antibiotic residues. More research is needed to find accurate field tests to determine violative residues on live dairy animals.

This project has established a substantial frozen tissue, urine, and serum bank from known untreated animals. This tissue bank could be of great value in testing the specificity of new tests as they become available.

Role of Industry

- Recognize the need to become proactive in pursuing animal production quality assurance program.
- Volunteer to participate in developmental meetings and cooperate in field studies to gather information needed to redesign the quality assurance program.
- Express commitment to develop and implement appropriate quality assurance programs.

Role of State and Federal Agency Personnel

- Support animal identification and traceback from slaughter.
- Take the responsibility to report slaughter test findings to producers and their veterinarians, improve the description of the extent of the residue problem, and gather the necessary information that can be used in finding a solution to the residue problem.
- With the assistance of dairy organizations, University of California Extension Service, veterinary schools, and regulatory agencies, facilitate meetings, document program components, and communicate with dairy producers.
- Measure the progress in reducing residues.
- Help identify changes needed in the quality assurance program to accomplish the goal of reducing residues.

Development and Support of the California Poultry Meat Quality Assurance Plan

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Purpose

The proposal for the development of a California poultry meat quality assurance program was first discussed with one large poultry meat company in California. This proposal is an offshoot of a preharvest food safety seminar held in Bethesda, Maryland in March 1994. Using the California Egg Quality Assurance Program (CEQAP) as an example, the California State Veterinarian presented the concept of preharvest food safety to the California Poultry Industry Federation (CPIF). Following this presentation, the Executive Secretary of CPIF requested APHIS to assist the Food Safety Committee of the California Department of Food and Agriculture (CFDA) Animal Health Branch in establishing a poultry meat quality assurance program in the state.

A series of meetings were held with the California poultry meat producers, California University Extension, private practicing veterinarians, the California Veterinary Diagnostic Laboratory System, and CDFA and APHIS veterinarians. Consideration was given to chemical and antibiotic residues, as well as potential microbial pathogens. The concept of Hazard Analysis Critical Control Points (HACCP) was included in the plan.

Discussion

Core Components

- **Administrative**
  - Develop a farm/premises flock poultry meat quality assurance plan.
  - Designate an employee or employees as the official quality control supervisor(s) for in-house operations and for follow-up training.
- **Production**
  - Purchase eggs or produce chicks, poults, ducks or game birds from breeders and hatcheries participating in the National Poultry Improvement Plan (NPIP), "U.S. Sanitation Monitored" or equivalent state plan as applicable by species.

* Transport live birds in vehicles that are decontaminated between deliveries to farms.
* Obtain feed from mills that follow accepted feed industry Good Manufacturing Practices and the Recommended Salmonella Control for Processors of Livestock and Poultry Feeds 1988 by the American Feed Industry Association (AFIA) or an equivalent program.
* If used, animal protein ingredients must originate from rendering plants participating in the Animal Protein Producers Industry (APPI) Salmonella Reduction Education Program or equivalent.
* If used, medications, feed additives and pesticides must be administered according to approved label directions or under veterinary supervision.
* Maintain an effective flock health program which can include appropriate vaccinations, monitoring, and periodic necropsy.
* Maintain a farm rodent monitoring and control program.
* Maintain a litter management program.
* Live haul vehicles, crates and equipment must be cleaned and disinfected between each flock.
* Live production buildings and equipment must be cleaned and disinfected on a routine basis.
* Utilize a biosecurity plan and train employees on proper procedures to execute the program on the farm.

The educational support for meeting the training requirements of the plan are being developed under the leadership of the University of California-Davis Extension Service, industry representatives, the California Veterinary Diagnostic Laboratory System, CDFA and APHIS veterinarians. The type of third party plan validation is under discussion. There will be an industry oversight committee selected to monitor the progress and needs for change of the program.

Preharvest Food Safety VMO Training

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Abstract

The Animal and Plant Health Inspection Service (APHIS) and the California Department of Food and Agriculture (CFDA) collaborated with the University of California-Davis School of Veterinary Medicine in the development of a preharvest food safety training program to meet the training needs of Veterinary Medical Officers (VMO's). The first VMO training course was designed in collaboration with UC-Davis researchers, extension managers, epidemiologists, and the Centers for Epidemiology and Animal Health (CEAH). The second course was designed to explore aspects of on-farm food safety, with special emphasis in dairy production.

California state and federal VMO's had been involved *Salmonella enteritidis* tracebacks in the egg industry and antibiotic residue tracebacks from slaughter. This training course was an attempt to meet both the current and potential future needs of federal and state VMO's in understanding preharvest food safety issues. There were many questions being raised about the contributions that could be made by producers to reduce the risk of foodborne pathogens. This training course was designed to debate those questions and bring the current state of technology and research to the VMO's.

The first VMO training course was designed in collaboration with University of California-Davis researchers, extension managers, epidemiologists, and a representative from CEAH. The first training was a two-week course included topics on the following: Healthy People 2000, preharvest food safety (i.e., Hazard Analysis and Critical Control Points), foodborne diseases (*i.e., E. coli* O157:H7, Salmonella), residues, and computer utilization.

The second course was designed to explore many facets of on-farm food safety with special emphasis in dairy production. Several speakers covered aspects of food safety which included such matters as the strengths and weaknesses of diagnostic assays, information on foodborne and waterborne pathogens, discussions of HACCP principles and methods, describing potential chemical and microbial residue hazards, and regulatory issues focused on public health concerns.

The course objectives were to: (1) train VMO's in techniques that can be used to reduce the risk of contaminating food supply with chemical or microbial residues, (2) provide additional training in scientific data collection and interpretation of diagnostic test results, and (3) train VMO's to help producers solve problems and enhance on-farm food safety capabilities.

Fifteen VMO's from CDFA Animal Health Branch and APHIS's Veterinary Services were trained in the first two-week course. The second one-week (condensed) course included 25 state and federal VMO's from the Western Region. The following western states sent VMO's to the training: Arizona, California, Montana, Oregon, and Utah.

Miscellaneous California Food Safety Initiatives

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California Cow-Calf Quality Assurance Program

The California Cattlemen's Association (CCA) has a comprehensive program called the Cow-Calf Quality Assurance Program in place. This program preceded the APHIS preharvest food safety initiatives. California Department of Food and Agriculture (CDFA) Animal Health Branch and Animal and Plant Health Inspection Service (APHIS) managers meet with the CCA Animal Health Committee at least twice a year. CCA has invited APHIS:VS to participate in their newly formed Food Safety Committee. CDFA and APHIS Veterinary Medical Officers participate or assist in conducting the CCA county level producer training for the Cow-Calf Quality Assurance Program in California. CDFA and APHIS monitor the new residues found by the Food Safety and Inspection Service (FSIS) on culled beef cows; however, it is very rare to find antibiotic residues in beef breeds at slaughter in California.

Participation in the National Pork Producer Quality Assurance Program

The California Pork Producers (CPP) and the California Farm Bureau (CFB) Swine Health Committees meet jointly at least twice a year. CDFA Animal Health Branch and APHIS managers have presented the preharvest food safety concepts to to the committees and offered assistance in any quality assurance efforts of these organizations. CPP and CFB currently participate at some level in the National Pork Producers Quality Assurance Program which deals primarily which antibiotic residues. California pork producers feel they have solved most of their antibiotic residue problem when they successfully eliminated sulfa residues a few years ago. CPP and CFB requested APHIS to monitor the pork residues through FSIS and keep them informed of the microbial sampling at slaughter and any impact it might have on consumer confidence and marketing issues.

California Wool Growers Approach to a Quality Assurance Program

The Animal Health Committee of the California Wool Growers Association meets at least twice a year. CDFA Animal Health Branch and APHIS managers meet with this committee regularly. This committee recently asked APHIS and CDFA to inform the committee of preharvest food safety concepts when the committee met to consider the National Sheep Associa-

tion's proposed quality assurance program. The Association decided to form a quality assurance working group. This group will develop a quality assurance plan for the California wool industry. APHIS and CDFA have been invited to participate in this working group.

Cooperative Service Agreement APHIS:VS and Wyoming Department of Agriculture State Veterinary Laboratory

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FSIS rules and regulations for state-inspected slaughter facilities required the training of meat inspectors and contract veterinarians in the essentials of meat inspection. Several years ago, the Wyoming Department of Agriculture requested the Wyoming Area Veterinarian In Charge to allow one of Veterinary Services' (VS) Veterinary Medical Officers (VMO's) to conduct the training of Wyoming lay meat inspectors and contract veterinarians.

Currently, a former FSIS employee who transferred to VS in Wyoming conducts the training. Training is accomplished through seminars, training aids, and hands-on experience. This same VMO is responsible for inspecting slaughter plants and making recommendations to the Wyoming Department of Agriculture. He makes sure that sampling is done and that samples are shipped in good condition to the appropriate laboratories.

The program is working and has increased the credibility of APHIS:VS in the eyes of the Wyoming Department of Agriculture. It is recommended that the cooperative service program be allowed to continue.

Miscellaneous Interagency Public Health Collaboration

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As the Animal and Plant Health Inspection Service' (APHIS) full-time liaison veterinarian with the Centers for Disease Control and Prevention (CDC), Dr. Thomas Gomez was involved in a variety of APHIS-CDC food safety and animal public health initiatives. This is a report on Dr. Gomez' activities in collaboration with CDC.

Activities

Provided federal (CDC, FDA) and state public agencies with data and information regarding APHIS animal health and preharvest activities/agenda.

- State contacts were typically with State/County Health Department epidemiologists/sanitarians, generally on a daily basis, to provide both epidemiology and APHIS/CDC program guidance and to obtain information related to APHIS program and food safety needs (e.g., *Salmonella enteritidis*, *E. coli* O157:H7, and tuberculosis).
- Provided information on issues of food safety (specifically SE and *E. coli* O157:H7) to the media, consumer advocate groups, industry, and state government personnel.
- Assisted in developing the CDC draft proposal titled, "The Epidemiology of Foodborne Disease at Sentinel Sites in the United States." This active surveillance project was funded by the Food Safety and Inspection Service (FSIS) and the Food and Drug Administration (FDA).
- Represented APHIS on the interagency working group developing parallel antimicrobial susceptibility monitoring systems in animals and humans.
- Assisted in the development of the monitoring (and baseline evaluation) for the animal system includes using Salmonella isolates from the National Animal Health Monitoring System (NAHMS), the National Veterinary Services Laboratory (NVSL), and National Animal Disease Center (NADC).

- Designing a study to evaluation the occupational risk of FSIS slaughter plant employees to zoonoses (brucella and TB).

Special Projects

- Drafted the "Prevention Program for SE," part of the CDC Fiscal Year 1995 document titled, "Prevention Program for Foodborne Diseases Epidemiology and Laboratory Sections."
- Co-author of the following papers to be submitted for publication:
  - "A mixed foodborne outbreak of *Salmonella heidelberg* and *Campylobacter jejuni* in a nursing home - New York City, 1993."
  - "A recurrent outbreak of *Salmonella enteritidis* phage type 4: lessons for prevention strategies."

Salmonella enteritidis Traceback Activities

Tracebacks are required for human outbreaks of *Salmonella enteritidis* associated with eggs. In Fiscal Year 1995, APHIS Veterinary Medical Officers and Area/Regional Epidemiologists conducted tracebacks in the following states:

State of Origin	State of Traceback	Comments
Virginia	Virginia	Not confirmed egg related
Indiana	Ohio	
Kentucky	Ohio	
Oregon	Oregon	

National Veterinary Services Laboratories Support of Food Safety Activities

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In Fiscal Year 1995, the National Veterinary Services Laboratories (NVSL) provided extensive diagnostic services in support of the Animal Production Food Safety Program (APFSP). The Cattle on Feed Evaluation (COFE) conducted by the National Animal Health Monitoring System (NAHMS) involved testing of 7,122 fecal samples for *Escherichia coli* O157:H7.

NVSL also provided support to the NAHMS Swine '95 Survey. This survey involved testing of 2,200 fecal samples for *E. coli* O157:H7 and 3,336 samples for Salmonella. To determine a rough prevalence of *Salmonella enteritidis* in laying hens, NVSL cultured more than 1,000 liquid egg samples. A repeat of the 1991 Spent Hen Survey was completed. This involved the serotyping of approximately 1,000 Group D Salmonella and phage typing of approximately 600 *S. enteritidis*.

The NVSL Salmonella Serotyping Service provided support to Animal Production Food Safety programs. Cultures came from a variety of sources. Over 120 Salmonella isolates were submitted by the National Animal Disease Center (NADC) as part of the COFE study. A total of 26 serotypes were identified from a total of 38 feedlots (out of 100) in this study.

Support was also provided to the Agricultural Research Service (ARS) laboratories investigating methods of controlling Salmonellae in poultry. ARS laboratories submitted approximately 500 Salmonella cultures. In addition, Food Safety and Inspection Service (FSIS) laboratories submitted approximately 550 Salmonella cultures in support of ot its food safety monitoring programs.